From the Department of Clinical Sciences, Danderyd Hospital
Division of Internal Medicine
Karolinska Institutet, Stockholm, Sweden

# CLINICAL ASPECTS ON GLUCOSE-LOWERING THERAPIES IN TYPE 2 DIABETES

Åsa Nybäck-Nakell


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*What lies behind us and what lies before us are*
*tiny matters compared to what lies within us*

Ralph Waldo Emerson

To my family

# ABSTRACT

Type 2 diabetes is a progressive disease with deterioration of glycaemic control over time in association with loss of endogenous insulin secretion. As a consequence of this, sulphonylureas (SU), which exert their mode of action by stimulating the pancreatic β-cells, are expected to be less effective with longer duration of diabetes. Thus SU is often withdrawn when insulin is started and SU is less frequently added to insulin and metformin in patients not reaching glycaemic goals. The aim of the work presented in this thesis was to investigate different aspects on the use of SU in patients with diabetes exceeding more than 5-10 years.

In **Study I** glycaemic control and β-cell function were monitored during a period of SU withdrawal in 25 patients**,** median diabetes duration 19 (8-29) years and on combined SU + insulin for > 5 years. In 80% glycaemic control deteriorated after SU withdrawal. Diabetes duration was positively correlated to the increase in fasting plasma glucose, i.e. in this group of patients a longer diabetes duration indicated more benefit of SU.

In **Study II** changes in $HbA_{1c}$ and β-cell function, assessed as C-peptide/glucose ratio, were observed at two time points, ten years apart, in patients who had attended the Diabetes Day Care Centre in 1997/1998. Of 462 patients, 171 attended the follow-up visit ten years later. Possible relations between SU treatment and changes in β-cell function were studied. $HbA_{1c}$ and β-cell function decreased but long-term use of SU was not associated with a more pronounced decline in β-cell function. It was concluded that these observations did not support the concept that SU is harmful to the β-cell.

**Study III** was performed to test whether SU still can be effective after > 10 years of diabetes. This randomized, placebo-controlled, double-blind, cross-over study included 43 patients, median diabetes duration 16 (10-30) years, with on-going metformin and insulin therapy. During two treatment periods of 12 weeks, separated by a washout period of six weeks, patients were given placebo/glimepiride in a randomized order as add-on therapy. No changes in baseline therapy were made except for insulin reduction if needed. $HbA_{1c}$ and changes in insulin requirement were primary outcomes. With glimepiride, $HbA_{1c}$ decreased from 7.0% to 6.4% (63-56 mmol/mol) while no change was observed during the placebo period. The insulin dose was reduced in 23 patients (median change 29%) with glimepiride addition. No severe hypoglycaemia occurred but 22 patients reported 124 minor hypoglycaemias, 74% of them occurring during the glimepiride period. Nocturnal glycaemia was monitored with CGMS at the end of each treatment period. In 15 patients on glimepiride episodes of glucose < 3.1 mmol/l were observed; and in six patients when placebo was added. It was concluded that addition of glimepiride to insulin + metformin therapy can lower $HbA_{1c}$ and reduce insulin requirement despite a long duration of diabetes.

In **Study IV** the accuracy of nocturnal CGMS monitoring was assessed after 48 and 72 hours in 14 patients on combined oral and insulin therapy. The study was conducted in the early era of CGMS and the sensor was the first model on the market. Nocturnal reference P-glucose values were assessed seven times and compared to sensor readings. In a Clark Error Grid model 100% of values were within zones A+B after 48 hours while only 44 % were in zone A and 7% in the unacceptable zone D after 72 hours. With the sensor used in the study the accuracy of CGMS thus deteriorated over time.

**Conclusion:** The decline in β-cell function over times varies considerably between patients. Sulphonylureas are effective when combined with insulin and metformin in many patients with long-standing type 2 diabetes; and long-term treatment with SU is not associated with are a pronounced decline in β-cell function.

# LIST OF PUBLICATIONS

I. Nybäck-Nakell Å, Adamson U, Lins P E, Landstedt-Hallin L
Glycaemic responsiveness to long-term insulin plus sulphonylurea therapy as assessed by sulphonylurea withdrawal.
*Diabetic Medicine, 2007; 24: 1424-29.*

II. Nybäck-Nakell Å, Bergström J, Adamson U, Lins P E, Landstedt-Hallin L
Decreasing postprandial C-peptide levels over time are not associated with long-term use of sulphonylurea: An observational study.
*Diabetes Metabolism, 2010; 36: 375-80.*

III. Nybäck-Nakell Å, Adamson U, Lins P E, Landstedt-Hallin L
Adding glimepiride to insulin + metformin in patients with type 2 diabetes of more than ten years' duration – a randomized, double-blind, placebo-controlled, cross-over study.
*In manuscript.*

IV. Nybäck-Nakell Å, von Heijne M, Adamson U, Lins P E, Landstedt-Hallin L
Accuracy of continuous nocturnal glucose monitoring (CGMS) after 48 and 72 hours in patients with type 2 diabetes on combined oral and insulin therapy.
*Diabetes Metabolism, 2004; 30: 517-21.*

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ACCORD | Action to control cardiovascular risk in diabetes |
| ADA | American Diabetes Association |
| ADOPT | A diabetes outcome progression study |
| ADVANCE | Action in diabetes and vascular disease: preterax- and diamicron-modified controlled evaluation |
| ATP | adenosine triphosphate |
| b.i.d. | [lat.] *bis in diae* = two times per day |
| BMI | body mass index |
| CGMS | continuous glucose monitoring system |
| C-peptide | connecting peptide |
| CV | coefficient of variation |
| CVD | cardiovascular disease |
| DCCT | Diabetes Control and Complications Trial |
| EASD | European Association for the Study of Diabetes |
| FPG | fasting plasma glucose |
| GAD | glutamic acid decarboxylase |
| $HbA_{1c}$ | glycosylated haemoglobin $A_{1c}$ |
| HOMA | homeostatic model assessment |
| IAPP | islet amyloid polypeptide |
| IFCC | International Federation of Clinical Chemistry and Laboratory Medicine |
| IGT | Impaired Glucose Tolerance |
| i.v. | intravenous |
| IVGTT | intravenous glucose tolerance test |
| JDS $HbA_{1c}$ | Japan Diabetes Society standardized glycohaemoglobin |
| LADA | latent autoimmune diabetes in adults |
| n/a | not available *or* not applicable |
| NGSP | National Glycohaemoglobin Standardization Program |
| NGT | normal glucose tolerance |
| NHANES | National Health and Nutrition Examination Survey |
| SMPG | self-monitoring of plasma glucose |
| SU | sulphonylurea |
| TLV | Tandvårds- och läkemedelsförmånsverket |
| TZD | thiazolidinediones |
| UKPDS | United Kingdom Prospective Diabetes Study |

# INTRODUCTION

Type 2 diabetes is one of the most common chronic diseases in the world and the number of people with the diagnosis continues to increase [Whiting et al. 2011]. It is estimated that 366 million people worldwide had diabetes in 2011 and it is assumed that by 2030 around 552 million people will have the diagnosis. Ninety percent of these people have type 2 diabetes and 80% live in low- and middle-income countries [IDF 2011].

Patients with diabetes risk developing both micro- and macrovascular complications [Zimmet et al. 2001] and persons with type 2 diabetes have a more than two-fold increased risk of death from cardiovascular diseases (CVD) than those without diabetes [Saydah et al. 2001]. The risk of complications is related to glycaemia, measured by $HbA_{1c}$, as has been shown in prospective studies both in type 1 [DCCT 1993] and type 2 diabetes [UKPDS 1998; Shichiri et al. 2000]

In the United Kingdom Prospective Diabetes Study (UKPDS) patients with newly diagnosed type 2 diabetes were allocated to conventional or intensive treatment with insulin or sulphonylurea (SU) and followed for no less than 10 years [UKPDS 1998]. The overall $HbA_{1c}$ achieved was 0.9% lower in the intensive-treatment group than in the conventional group (7.0% vs. 7.9%), and this was associated with a significant reduction of microvascular complications and a trend towards a reduction of macrovascular complications. In addition, epidemiological analyses from UKPDS revealed that the risk of complications was strongly related to previous hyperglycaemia and that any reduction in $HbA_{1c}$ of 1%, regardless of starting value, was associated with a 21% decrease in the risk of any diabetes-related endpoint [Stratton et al. 2000].

In 2008 two large trials - ACCORD [Gerstein et al. 2008] and ADVANCE [Patel et al. 2008] - were presented. They both sought to further study whether intensive glucose control could reduce the risk of CVD events in patients with type 2 diabetes. However, neither study showed that lowering $HbA_{1c}$ below 7%, i.e. IFCC 53 mmol/mol, was beneficial in reducing macrovascular complications or mortality; indeed, mortality actually increased in the intensive group in the ACCORD study. A Cochrane review published in 2011 concluded that intensive glycaemic control had no significant effect on all-cause and cardiovascular mortality but reduced the risk of microvascular complications [Hemmingsen et al. 2011]. However, a long-term follow-up of the patients who had participated in the UKPDS demonstrated that, despite an early loss of the difference in $HbA_{1c}$ between conventional and intensive treatment groups after the end of study, there were emergent and significant reductions in myocardial infarction and total mortality for those receiving intensive therapy from start [Holman et al. 2008]. A continued reduction of microvascular risk was also confirmed in this analysis. These observations have been referred to as a legacy effect, indicating that intensive glucose control starting at the time of diagnosis is associated with a decreased risk of diabetes complications, emphasizing the importance of early and stringent interventions in the treatment of type 2 diabetes.

Even if treatment of hyperglycaemia is important, especially to reduce microvascular complications, multifactorial treatment addressing not only hyperglycaemia but also hypertension and

hyperlipidaemia, seems to be very important for protection against vascular complications, as was shown in the Steno-2 study [Gaede et al. 2008].

Type 2 diabetes is a progressive disease with a worsening of glycaemia over time in most patients. The proposed underlying mechanism is a continuing decline in β-cell function resulting in a loss of insulin secretory capacity [UKPDS 1995; Levy et al. 1998]. This induces a need for intensification of glucose-lowering therapies [Turner et al. 1999; Koro et al. 2004], which was successful as shown in a recent observational study where increased treatment with oral drugs and insulin maintained glycaemic control over five years [Best et al. 2012].

When intensifying glucose-lowering therapy with insulin, one problem is the risk of hypoglycaemia [Zhang et al. 2009; Best et al. 2012]. This is also true but to a smaller extent for SU [Leese et al. 2003]. Hypoglycaemia has received limited attention as a problem in type 2 diabetes, but there are reasons to question this [Frier 2002]. First, the risk of hypoglycaemia increases with longer diabetes duration [Henderson et al. 2003]. Secondly, there is currently much emphasis on adherence to strict glycaemic goals in type 2 diabetic patients with an increased use of insulin. This in turn increases the frequency of hypoglycaemia [Donnelly et al. 2005]. Thirdly, since the prevalence of type 2 diabetes escalates with increasing life expectancy, the number of older people with insulin-treated diabetes will increase [Whiting et al. 2011].

Insulin secretagogues, such as SUs, are effective glucose-lowering agents [Inzucchi 2002] but their long-term effectiveness has been questioned since they depend on a residual β-cell function for mechanism of action [DeFronzo 1999] and there is a strong association between glycaemic deterioration and progressive β-cell failure in type 2 diabetes.

The main focus of these studies was issues related to the use of sulphonylureas in the treatment of patients with diagnosed type 2 diabetes of more than 5-10 years' duration.

# BACKGROUND

## A PROGRESSIVE DISEASE

The UKPDS showed that the deterioration in glycaemic control observed over time in patients with newly-diagnosed type 2 diabetes is associated with a decrease in β-cell function [UKPDS 1995]. Other long-term follow-up studies reported a decrease in insulin secretion over time [Niskanen et al. 1994; Levy et al. 1998] and in cross-sectional studies lower insulin secretion has been associated with longer diabetes duration [Clauson et al. 1994; Shim et al. 2006; Funakoshi et al. 2008]. However, chronic hyperglycaemia itself contributes to impaired insulin secretion by making the β-cell unresponsive to glucose – the glucotoxicity effect [Leahy et al. 1992]. This may affect the observed decrease in insulin secretion, since glycaemic control deteriorated with time as shown in those studies.

Although it seems clear that β-cell function declines over time in patients with type 2 diabetes as a group, the disease is probably heterogeneous with respect to decreasing β -cell function, and the loss of insulin secretory capacity may not be inexorable [Zangeneh et al. 2006]. Two long-term follow-up studies have, in contrast to others, reported that insulin secretory capacity can be preserved for up to twenty years in some patients [Zangeneh et al. 2006; Ekholm et al. 2012]. Notably, in these studies glycaemic control improved as a result of more intensive treatments.

In an analysis from the UKPDS assessing the number of patients attaining glycaemic goals on monotherapy after nine years, only approximately 25% did so, and the conclusion was that the majority of patients need multiple therapies with both oral agents and insulin to attain glycaemic targets in the long run [Turner et al. 1999]. Using multiple therapies it is possible to minimize the progressive deterioration in $HbA_{1c}$ as was shown in the Action in Diabetes and Vascular Disease: Preterax- and Diamicron-modified Controlled Evaluation study (ADVANCE), in which $HbA_{1c}$ decreased over five years [Patel et al. 2008].

Longitudinal data regarding glucose-lowering treatments and glycaemic control in patients with type 2 diabetes from the U.S. National Health and Nutrition Examination Survey (NHANES, 1988-2000 and 1999-2004) show that treatment regimens have changed substantially over fifteen years, with a marked increase of combined oral glucose-lowering agents and insulin [Koro et al. 2004; Ong et al. 2008]. With this combined therapy, glycaemic control improved between 1999 and 2004, and $HbA_{1c}$ decreased from 7.9% to 7.2% (IFCC 63-55 mmol/mol) and 52% of patients had an $HbA_{1c}$ < 7.0% (53 mmol/mol) [Ong et al. 2008].

### β-cell failure

The pathogenesis of type 2 diabetes is complex, involving both genetic and environmental factors. In a non-diabetic person β-cells can balance changes in insulin sensitivity with proportionate changes in insulin secretion, thus keeping blood glucose at normal levels [Kahn et al. 1993]. In a person with type 2 diabetes a combination of reduced β-cell mass and decreased function together

with insulin resistance results in β-cells incapable of compensating adequately for increased secretory demand due to insulin resistance [Lyssenko et al. 2005; Karaca et al. 2009]. When the β-cell compensation becomes insufficient, early in the course of the disease, a mild increase in glycaemia occurs when moving from normal to impaired glucose tolerance, progressing to overt diabetes [Leahy et al. 2010], see figure 1.

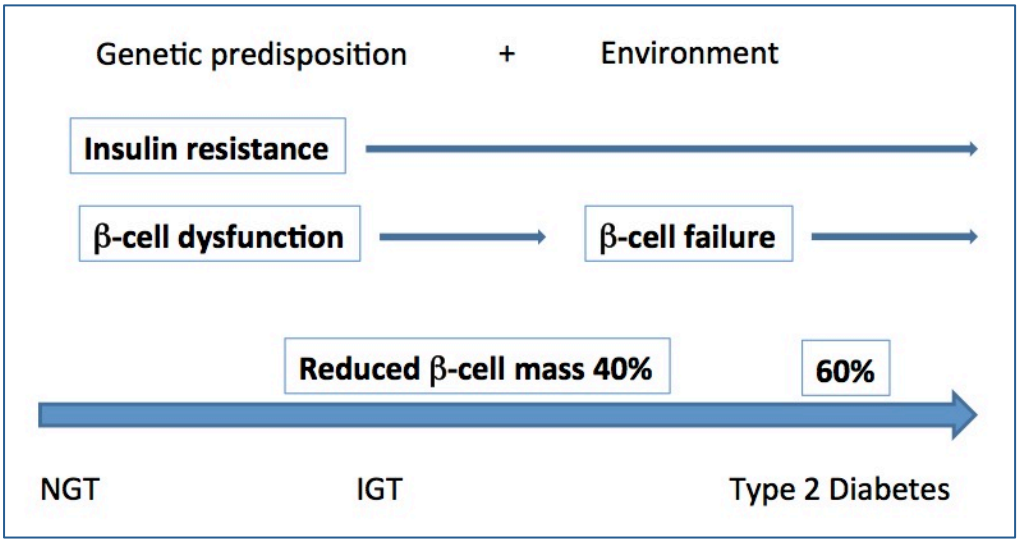


**Figure 1**. Key pathological features of type 2 diabetes.
*Adapted with permission from J.L. Leahy; Arch Med Res 36:197-209, 2005.*

The initial increase in glycaemia causes further deterioration in β-cell function through a number of proposed mechanisms such as gluco- and lipotoxicity. These contribute to the progressive impairment of insulin secretion and lower insulin sensitivity while hyperglycaemia progresses [Unger 1995; Poitout et al. 2002]. Other acquired factors that may contribute to β-cell failure or death are increased deposition of islet amyloid polypeptide (IAPP) [Butler et al. 2003] and inflammatory cytokines [Donath et al. 2005]. In combination, all these factors interact on the β-cell, leading to decreased function and lowered mass. Besides the β-cell defects there are various degrees of insulin resistance early in the course of the disease; but it is the worsening of β-cell function and insulin secretion that is believed to be responsible for the progressive rise in glycaemia [Weyer et al. 1999].

## Methods for evaluating insulin secretion and β-cell function

The homeostasis model assessment, HOMA [Matthews et al. 1985], is the best known and validated method [Hermans et al. 1999] for assessing insulin secretion. The HOMA-B estimates β-cell function with a mathematical formula involving fasting plasma insulin (or C-peptide), and glucose concentrations. The original formula can be written:

$$\text{HOMA-B} = \frac{20 \times \text{fasting P-insulin } (\mu\text{U/ml})}{\text{FPG (mmol/L)} - 3.5}$$

In 2004 a HOMA calculator was released online which provides model-derived estimates rather than linear approximations. As insulin is part of the formula, the model cannot be used in patients

taking exogenous insulin. It does not provide information on the dynamic relationship between insulin sensitivity and secretion.
An oral glucose tolerance test (OGTT) is easy to perform and, besides its use for diagnosing impaired glucose tolerance or diabetes, it can be used to assess β-cell function. The measure most commonly examined, after 75 g oral glucose load, is the early insulin response, the insulinogenic index, which is determined as the ratio of the incremental insulin (or C-peptide) to glucose (Δinsulin/Δglucose) at 30 min [Haffner et al. 1995]. This can be followed by frequent sampling for up to two hours [Kahn et al. 2008]. Instead of oral glucose a meal tolerance test with frequent sampling can be performed [Mari et al. 2002].

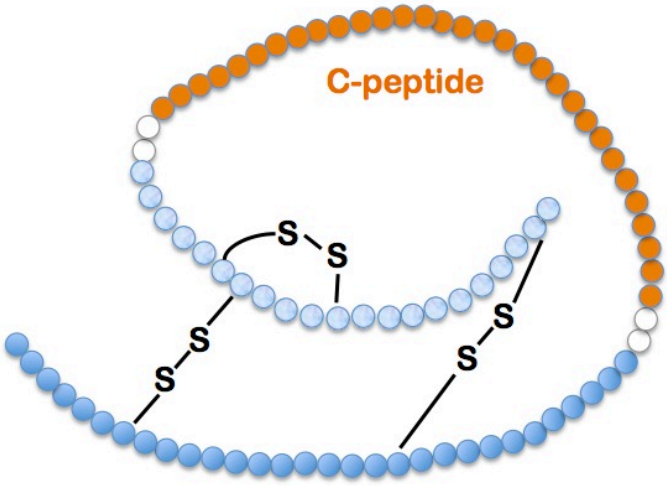


**Figure 2.** Human proinsulin. B-chain (dark blue) and A-chain (light blue) are linked by C-peptide (orange).

C-peptide is co-secreted by the pancreas in an equimolar ratio with insulin and has been used as a tool for assessing insulin secretion (Fig. 2). Unlike insulin, C-peptide is not cleared by the liver, making it a more suitable peptide for assessing endogenous insulin secretion than insulin [Hovorka et al. 1998]. Measuring C-peptide after stimulation with intravenous glucagon provides a reliable estimate of β-cell function [Gjessing et al. 1987] and has been widely used. Another option is the standard breakfast test since there is a good correlation between C-peptide increments in these two tests [Koskinen et al. 1988; Escobar-Jimenez et al. 1990].

Indexes for evaluation of fasting and postprandial β-cell responsiveness by using C-peptide after a standard meal have been developed [Hovorka et al. 1998]. Fasting β-cell responsiveness is the ratio of fasting C-peptide/glucose. Postprandial β-cell responsiveness represents the ability of postprandial glucose to stimulate C-peptide secretion. It corresponds to the increment in postprandial C-peptide divided by the increment in postprandial glucose (ΔC-peptide/Δglucose).

In addition to the tests described above, various experimental methods for measuring insulin secretion and insulin sensitivity have been developed. The gold standard is the glucose clamp technique, originally described by DeFronzo et al [DeFronzo et al. 1979]. This can be done as a hyperglycaemic clamp when the plasma glucose level is raised by intravenous glucose infusion and held constant. During the constant hyperglycaemia, insulin secretory response is measured.

Another option is the euglycaemic insulin clamp, when insulin is infused at a constant rate and the amount of glucose infused to maintain glucose at a constant level serves as a measure of insulin sensitivity. A further test used in experimental settings is the intravenous glucose tolerance test (IVGTT), in which the acute insulin response 30 minutes after a glucose bolus injection is measured and then followed by frequent sampling for up to 4 hours [Brunzell et al. 1976].

While the intravenous tests provide detailed information on the dynamic state of the relationship between insulin secretion and sensitivity, they are far too complicated to be used in a clinical setting or in larger clinical studies. A review article from 2004 concluded that there is no single test that allows β-cell function to be assessed with accuracy and specificity because of the enormous complexity of β-cell behaviour in vivo [Ferrannini et al. 2004]. Generally, since there is no consensus on a reference method, the results from various tests should be interpreted in the context of actual glucose levels and insulin sensitivity, especially when using more simple tests.

## GLYCAEMIC GOALS

Data from recent intervention studies in type 2 diabetes [Gerstein et al. 2008; Patel et al. 2008; Duckworth et al. 2009] suggested that not everyone benefits from intensive glucose management. This has resulted in a re-examination of the rigorous glycaemic targets for all patients, and the benefit of an intensified glucose control has been questioned [Yudkin et al. 2010].

In 2012 the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) published a position statement emphasizing the importance of a patient-centred approach to the management of hyperglycaemia [Inzucchi et al. 2012]. According to this document glycaemic targets in type 2 diabetes should be individualized depending on age, diabetes duration, presence of diabetes complications and propensity for hypoglycaemia. Further, the patient's capacity for self-care, living conditions, cognitive status and overall prognosis should be taken into consideration. Finally, glycaemic targets should be flexible, adapted to changes in the patient's health conditions; and the patient should be an active participant in setting goals [Ismail-Beigi et al. 2011].

Glycosylated haemoglobin, $HbA_{1c}$, is a measurement that reflects the average plasma glucose concentration over a period of up to12 weeks. It is used for setting glycaemic goals and evaluating glycaemic control. There has been no standardized method for measuring $HbA_{1c}$ but the most common world-wide has been the DCCT aligned assays. These are managed through the National Glycohaemoglobin Standardization Program, NGSP, [Little et al. 2011] with a reference value $\leq 6.0\%$. In Sweden $HbA_{1c}$ has been measured with the Mono-S method with a reference value of $\leq 5.2\%$. The NGSP/DCCT standard is approximately 0.9% higher than the Mono-S. In 2010 a new worldwide general standard for $HbA_{1c}$ was agreed upon by major diabetes organizations [Hanås et al. 2010] after recommendations from a working group in the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). $HbA_{1c}$ results are now to be reported in IFCC units (mmol/mol) and derived NGSP units (%) [IFCC 2007].

In Swedish guidelines for the management of diabetes from the National Board of Health and Welfare [Socialstyrelsen 2010] the recommended overall treatment goal is an $HbA_{1c}$ level of < 52 mmol/mol, (< 6.0% Mono-S or < 6.9% NGSP). However, the guidelines state that risks versus benefits for each patient should be assessed to individualize the glycaemic goal.

## PHARMACOLOGICAL TREATMENT

Lifestyle intervention is the basis for treatment of type 2 diabetes, but pharmacological treatment of hyperglycaemia is needed in almost all patients sooner or later due to the progressive nature of the disease. Figure 3 gives an overview of all classes of oral glucose-lowering drugs available on the market in August 2012.

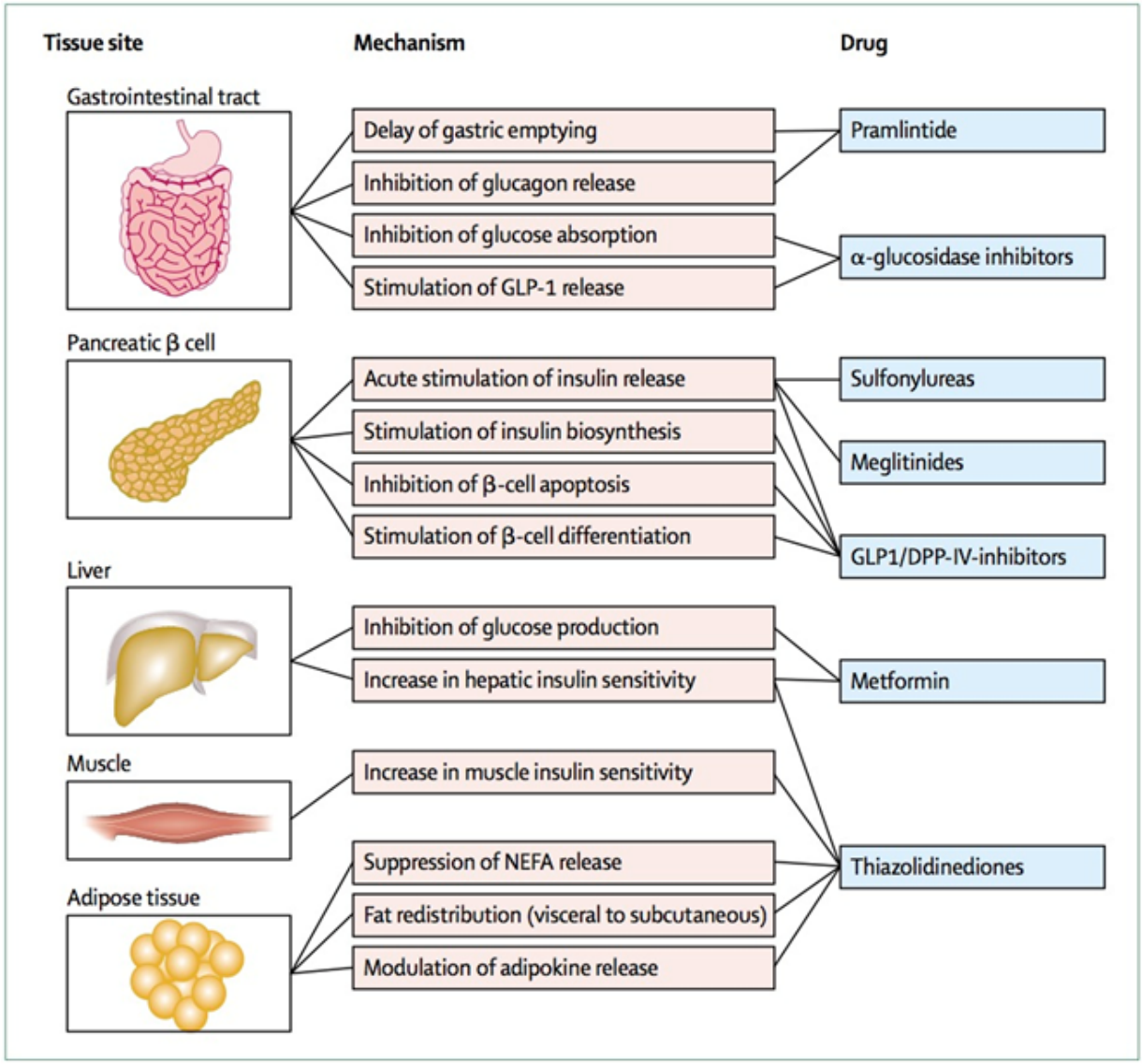


**Figure 3.** Pharmacological treatment of type 2 diabetes according to site of action.
*Published with permission from Stumvoll et al. Type 2 diabetes: principles of pathogenesis and therapy. Lancet 2005. 365: p. 1333-46.*

Metformin is considered in most guidelines to be the first choice of oral glucose-lowering medication [IDF 2006; Home et al. 2008; Nathan et al. 2009; Inzucchi et al. 2012]. However, what to add after metformin when glycaemic control deteriorates is more difficult to agree upon, mainly because of a lack of good clinical comparison studies of different drugs [Bennett et al. 2011]. Some guidelines recommend SU as second-line treatment after metformin [IDF 2006; Home et al. 2008]. In general, the addition of a second oral drug is, on average, associated with a reduction of $HbA_{1c}$ of approximately 1% i.e. IFCC 11 mmol/mol [Bennett et al. 2011]. Insulin is the most effective drug for lowering glucose and should be added relatively early, e.g. if $HbA_{1c}$ rises ≥ 7.5% (NGSP; IFCC > 58 mmol/mol) [IDF 2006; Home et al.2008]. Insulin can, when used in adequate doses, decrease any level of elevated $HbA_{1c}$ to, or close to, the therapeutic goal [Nathan et al. 2009].

The evidence basis for how to combine different glucose-lowering agents, and in what order they should be added, is lean. Data from clinical studies shows median responses and does not address the important question of who responds to which therapy and why [Smith et al. 2010]. The uncertainties in terms of which drug to choose, how to combine and when, for treatment of type 2 diabetes have resulted in a more patient-centred care [Glasgow et al. 2008]. Consequently, in the recent position statement from ADA/EASD on the management of hyperglycaemia the recommendations are "less prescriptive and not as algorithmic as prior guidelines", manifested in suggestions such as the following: if the $HbA_{1c}$ target is not achieved in three months, add either a second oral agent, a GLP-1 receptor agonist or insulin; the choice should be based on patient- and drug-specific characteristics with the primary goal to improve glycaemic control while minimizing side effects [Inzucchi et al. 2012]. Individualization is thus the cornerstone of success and the advantages and disadvantages of specific drugs for each patient should be considered when seeking the optimal therapy for a given patient [Inzucchi et al. 2012].

Since type 2 diabetes affects so many people, costs are a critical issue when choosing glucose-lowering therapy. Access to many of the options for treatment is limited in many middle- and low-income countries and less expensive agents are to be used [Colagiuri 2012].

## Sulphonylureas

Sulphonylureas have been used for treating type 2 diabetes since the mid-1950s. These drugs enhance insulin secretion [Gotfredsen 1976] by binding to a SU receptor on the surface of the β-cell. This leads to a closure of potassium adenosine triphosphate ($K_{ATP}$) channels followed by an opening of calcium channels, which in turn triggers insulin secretion [Zimmerman 1997]. These ATP-sensitive potassium channels are also found in the myocardium, in skeletal and smooth muscle [Ashcroft et al. 1992]. The clinically most important adverse effects of SUs are hypoglycaemia and weight gain [UKPDS 1998; Inzucchi 2002].

The currently available SU compounds glibenclamide (glyburide in the U.S.), glipizide, gliclazide (not available for prescription in Sweden) and glimepiride are associated with similar reductions in $HbA_{1c}$ levels of 1-1.5 % i.e. IFCC 11-16 mmol/mol [Clark et al. 1998; Sherifali et al. 2010]. Despite their similar glucose-lowering efficacy there are some clinically important differences between the various SUs. Thus gliclazide, glipizide and glimepiride have less adverse effects than

glibenclamide and hypoglycaemia frequency is clearly highest with glibenclamide [Rendell 2004] while gliclazide and glimepiride have a lower frequency, reportedly the same for both compounds [Tsumura 1995].

Glibenclamide has long been the most used SU compound worldwide, but its continued use has been questioned because of the safety concerns linked to this drug compared to the other SUs [Riddle 2010]. One major concern is that glibenclamide is associated with a greater risk of hypoglycaemia than the other SUs are [van Staa et al. 1997] and hence glibenclamide is no longer subsidized in Sweden for new patients as decided by the Dental and Pharmaceutical Benefits Agency [TLV 2010]. Another concern is related to a possible deleterious effect on cardiovascular events because of a negative effect of glibenclamide on what is termed myocardial ischemic preconditioning [Engler et al. 1996; Leibowitz et al. 1996].

Observational studies, mostly retrospective, of mortality and cardiovascular risk associated with the use of SU have reported conflicting results [Schramm et al. 2011; Pantalone et al. 2012]. In studies suggesting a poorer outcome with SU therapy, glibenclamide was the most commonly used SU, but the results were assumed to be the same for all different SUs [Evans et al. 2006; Simpson et al. 2006]. A recent, prospective, study aiming to determine whether SUs in general, and glibenclamide in particular, alter the risk of death or other in-hospital outcomes in patients with acute myocardial infarction, showed no increased mortality risk in patients on chronic SU therapy [Zeller et al. 2010] However, the risk of early mortality was significantly lower with glimepiride or gliclazide than with glibenclamide. The result is consistent with the findings that the newer classes of SUs do not interact with $K_{ATP}$ channels in the heart. When gliclazide-based therapy was used in the large, prospective, ADVANCE study, there was a significant reduction in microvascular events in the intensively-treated group and this was achieved with a low frequency of hypoglycaemia and no increase in CVD mortality [Patel et al. 2008].

Glimepiride was introduced 1996 as the most recent SU compound on the market. It is one of the recommended SUs in Sweden along with glipizide [Läkemedelsverket 2010]. Glimepiride has pharmacological properties that may be advantageous when compared to other SUs [Korytkowski 2004]. It is associated with lower rates of hypoglycaemia than glibenclamide [Dills et al. 1996; Holstein et al. 2001]. This could be because it has a lower binding affinity for the β-cell SU receptor [Kramer et al. 1996] and because lower levels of insulin and C-peptide are produced by glimepiride at the same level of glycaemia [Raptis et al. 1999]. The avoidance of excess insulin concentrations with glimepiride could also play a role for the weight neutrality or even weight loss that has been associated with glimepiride therapy [Bugos et al. 2000; Scholz et al. 2001; Weitgasser et al. 2003]. Glimepiride is quite exclusively specific to the pancreatic β-cells and reportedly does not induce negative effects on ischemic preconditioning in experimental myocardial ischemia [Klepzig et al. 1999]. The elimination of glimepiride in patients with renal impairment is not changed, making it a safer option in these patients [Rosenkranz et al. 1996].

The fact that SUs lose their efficacy over time or, more precisely, that there is a successive need for multiple therapies due to declining β-cell function has raised the concern that SU may exhaust β-cell function. This opinion emerges from studies indicating that glibenclamide can accelerate β-cell apoptosis in human β-cell islets [Maedler et al. 2005]. Other mechanisms proposing that SU may be deleterious for β-cells are that islet amyloidosis observed in patients with type 2 diabetes

is related to the degree of β-cell impairment in humans [Westermark et al. 2011]. Amyloidosis is formed primarily by deposition of islet amyloid polypeptide (IAPP) which, has been proposed, to be involved in the development of β-cell dysfunction [Clark et al. 1996]. There is experimental evidence for an association between elevated IAPP secretion, SU treatment and islet amyloidosis [Hoenig et al. 2000]. A third hypothesis, put forward in one clinical study [Pfutzner et al. 2006], is that an increased secretion of proinsulin, which is viewed as a symptom of a stressed β-cell [Hostens et al. 1999], is associated with SU therapy.
In the Diabetes Outcome Progression Study (ADOPT) [Kahn et al. 2006] glycaemic durability and changes in β-cell function were studied over four years in patients allocated to rosiglitazone, metformin or glyburide. The study showed a more rapid decline in insulin secretion and glycaemic control with glyburide, and the authors conclude that it was the more rapid loss of β-cell function with glyburide that led to the increasing FPG and $HbA_{1c}$ in patients allocated to this drug.

## Why SU and insulin?

Adding SU to metformin, the first drug of choice in the treatment of type 2 diabetes, is one of the options when glycaemic control deteriorates and a second drug is needed [Inzucchi et al. 2012]. However, the benefits of combining an insulin secretagogue, such as SU, with exogenous insulin are not as obvious [Raskin 2008].

The idea of combining insulin and SU originally came from early reports suggesting that SUs were thought to have extra-pancreatic effects leading to increased peripheral and hepatic insulin sensitivity [Melander et al. 1990] – in addition to stimulating endogenous insulin secretion. However, *in vivo* studies have not confirmed such effects of SUs [Gutniak et al. 1987] and their proposed peripheral effects are most likely secondary to a reduction in glucotoxicity. In the mid-1980s, when metformin was not available in the US, combining SU with bedtime insulin was a therapy regimen introduced [Riddle et al. 1984]. In the 1990s three meta-analyses of combined SU and insulin therapy were published [Peters et al. 1991; Pugh et al. 1992; Johnson et al. 1996]. They concluded that, compared to insulin monotherapy, combination therapy improved glycaemic control with fewer exogenous insulin doses. Many investigators have documented the efficacy of insulin and SU therapy, showing better $HbA_{1c}$ with combination therapy [Shank et al. 1995; Feinglos et al. 1998; Janka et al. 2007; Ebato et al. 2009].

One advantage of combining SU and insulin is that the insulin doses often can be reduced. In a review [Yki-Järvinen 2001] of 22 studies comparing combination therapy with insulin monotherapy, the insulin dose was, in mean, 42% lower with combination SU and insulin. The insulin-sparing effect of SU in combination therapy was also the conclusion from the earlier meta-analyses [Peters et al. 1991; Pugh et al. 1992; Johnson et al. 1996]. Another possible advantage is that the increase in body weight associated with improved glycaemic control [Mäkimattila et al. 1999] may be somewhat smaller with combined insulin and SU than with insulin monotherapy for the same level of glycaemia [Chow et al. 1995; Landstedt-Hallin et al. 1995; Clauson et al. 1996; Riddle et al. 1998; Yki-Järvinen et al. 1999]. A possible disadvantage of combining an insulin secretagogue with insulin could theoretically be an increased risk of hypoglycaemia. However, studies have suggested that combination therapy is accompanied by a similar [Yki-Järvinen 2001] or lower [Kabadi et al. 2003; Goudswaard et al. 2004; Janka et al. 2005] frequency of hypo-

glycaemia. One explanation could be the insulin-sparing effect of SUs since endogenous insulin probably has physiological benefits compared to exogenous insulin [Lebovitz 2011]; and when more of the glycaemic control is achieved with endogenous insulin, glycaemic stability may improve [Riddle et al. 1992].

As SUs stimulate insulin secretion they depend on residual β-cell function and, given the progressive nature of type 2 diabetes, they would be expected to be less effective over time; but few studies evaluate the long-term efficacy of combined SU + insulin therapy. In the ADVANCE study patients in the intensive treatment group, in which approximately 50% were on combined gliclazide + insulin therapy, achieved $HbA_{1c}$ of 6.5% (NSGP; Mono-S 5.6%, IFCC 48 mmol/mol) after a median follow-up of five years. A sub-study of the UKPDS [Wright et al. 2002], which used a modified protocol for the last UKPDS centres, allowed the addition of insulin in patients allocated to SU if maximal doses of SU did not maintain FPG < 6.0 mmol/l. After six years 52% of patients required combination therapy, and SU + insulin resulted in a lower $HbA_{1c}$ than in the insulin monotherapy group. A clinically important question is clearly whether SU remain effective in patients with long diabetes duration.

## HYPOGLYCAEMIA IN TYPE 2 DIABETES

There is no consensus on a common definition of biochemical hypoglycaemia [Service 1995] and thus definitions based on symptomatology have been adopted. Mild hypoglycaemia is defined as an event where symptoms suggestive of hypoglycaemia are successfully treated by the patient alone, while episodes requiring third-party assistance are defined as severe [Zammitt et al. 2005].

Symptoms of hypoglycaemia are usually classified as autonomic, neuroglycopenic and non-specific. Elderly people report a profile of hypoglycaemic symptoms that partly differs from that of young adults, and the intensity of their symptoms is less [Brierley et al. 1995]. In young adults, symptoms of hypoglycaemia are generated at a glucose level that is around 1 mmol/l higher than the level at which cognitive function becomes impaired [Schwartz et al. 1987], while in elderly subjects these thresholds are much closer together, allowing less time for remedial action [Matyka et al. 1997]. Moreover, in elderly diabetes patients, neurological symptoms affecting vision and co-ordination have been identified as more frequent and may be confused with other conditions such as a transient ischemic attack or a vasovagal episode [Jaap et al. 1998; McAulay et al. 2001]. The risk that a hypoglycaemic episode is not correctly identified and treated in elderly type 2 patients is also magnified by the fact that many of these patients and their relatives have limited knowledge of hypoglycaemic symptoms [Mutch et al. 1985; Pegg et al. 1991]. Notably, a recent UK prospective survey documented that while only 10% of episodes of severe hypoglycaemia affecting type 1 patients required the assistance of emergency medical services, one in three of type 2 patients affected needed to enlist help from such services [Donnelly et al. 2005]. Impaired awareness of hypoglycaemia, which is a prominent risk factor for severe hypoglycaemia in type 1 diabetes, [Bragd et al. 2003] is less common in type 2 diabetes, affecting only 8 % according to one retrospective survey in insulin-treated type 2 patients [Henderson et al. 2003].

Most studies concerning the frequency of hypoglycaemia in type 2 diabetes patients have been conducted retrospectively and the definitions of hypoglycaemia and the nature of the treatment modalities examined both differ between studies thus hindering comparison [Zammitt et al. 2005]. Reported prevalence figures concerning severe hypoglycaemia in type 2 diabetes range from 2.3% [UKPDS 1998] to 16.5% in insulin-treated patients as revealed by a retrospective questionnaire [Akram et al. 2006]. The prevalence of severe hypoglycaemia in patients with type 2 diabetes appears to increase with duration of insulin therapy [Henderson et al. 2003], and this is in line with what is well-known amongst patients with type 1 diabetes [Bragd et al. 2003].

## CONTINUOUS GLUCOSE MONITORING

The first continuous glucose monitoring product, the CGMS® System, was introduced for monitoring glucose in 2000 by Medtronic Minimed. CGMS is a Holter-type sensor system that continuously measures interstitial glucose levels, usually in subcutaneous tissue of the abdomen. The sensor is a microelectrode that generates an electrical signal of which the strength is proportional to the glucose concentration in the fluid. The system generates a value every 5 minutes, 288 readings per day, measuring glucose levels between 2.2 and 22.2 mmol/l. It has to be calibrated with capillary plasma glucose 3-4 times every 24 hours. In the second-generation CGMS Gold™, launched in 2003, like in the initial CGMS, the current glucose values are not shown for the person being monitored and the system can only be used for retrospective analysis. The newer CGMS systems monitor interstitial glucose in real time and are most commonly used in clinical practice.

The advantages of CGMS are that it provides more data on glycaemic excursions than obtainable by self-monitoring of plasma glucose (SMPG) and that it gives the opportunity to monitor nocturnal profiles of glucose. The system was initially used for detecting unrecognized nocturnal hypoglycaemias in type 1 patients [Boland et al. 2001; Chase et al. 2001]. Although CGMS has been used less in patients with type 2 diabetes, studies suggest a high frequency of hypoglycaemic episodes, mostly unrecognized, during nighttime. The conclusion from these studies has generally been that CGMS is a useful tool to assess nocturnal glycaemia [Hay et al. 2003; Zick et al. 2007].

CGMS may be a valuable tool for improving glycaemic control [Klonoff 2005] and avoiding hypoglycaemia [Garg et al. 2006]. To provide clinical benefits it is important that sensor readings adequately reflect actual plasma glucose and for this reason sensor accuracy is important. The agreement between sensor readings and meter values (calibration values) has been acceptable in several studies [Gross et al. 2000; Sachedina et al. 2003; Kubiak et al. 2004].

It was suspected that the first generation CGMS overread nocturnal hypoglycaemia because of a high incidence of asymptomatic low glucose values [Klonoff 2005]. A study from 2002 reported spurious hypoglycaemic readings during nighttime when compared to simultaneously measured glucose reference values [McGowan et al. 2002].

# AIMS

- to assess the effect of sulphonylureas (SU) in patients on SU + insulin therapy by withdrawal of SU, and to identify clinically useful markers of long-term response *(Study I)*

- to describe long-term changes in glycaemic control and β-cell function, assessed as postprandial C-peptide and C-peptide/glucose ratio, in relation to treatment with SU over a period of 10 years *(Study II)*

- to assess the effect of SU in patients on metformin + insulin therapy – assessed as $HbA_{1c}$ and by CGMS – by adding glimepiride, and to identify predictors of long-term responsiveness to SU *(Study III)*

- to study the accuracy of continuous nocturnal glucose monitoring (CGMS) in patients with type 2 diabetes on combined oral and insulin therapy *(Study IV)*

# PATIENTS AND METHODS

## OVERVIEW

### Study characteristics

All participants were recruited either from the Diabetes Day Care Centre at Danderyd University Hospital, or through contacts with primary care physicians in the Stockholm area or through newspaper advertisements. Table 1 summarizes the characteristics of the studies while Table 2 summarizes the inclusion criteria for **Studies I+III**.

**Table 1.** Summary of study characteristics

| | Number of subjects | Study performed | Study design | Outcome |
|---|---|---|---|---|
| **Study I** | 25 | 2003–2005 | Open, prospective, observational | Fasting P-glucose, $HbA_{1c}$ |
| **Study II** | 171 | 2006–2007 | Observational | Ratio postprandial C-peptide/ glucose and $HbA_{1c}$ related to use of SU |
| **Study III** | 43 | 2010-2012 | Randomized, placebo-controlled, prospective, cross-over | $HbA_{1c}$<br>Insulin doses<br>Frequency of hypoglycaemia |
| **Study IV** | 14 | 1999-2003 | Prospective, pilot | Correlation between CGMS readings and venous P-glucose |

**Table 2.** Inclusion criteria for **Studies I and III**

| | Study I | Study III |
|---|---|---|
| **Age** | *No limitations* | 40-75 |
| **Diabetes duration** | ≥ 5 years with combined SU + insulin± metformin | ≥ 10 years treatment with metformin + insulin ≥ 1 year prior to inclusion |
| **$HbA_{1c}$** | < 8.5% (IFCC < 78 mmol/mol) | 6-8.5% (IFCC 52-78 mmol/mol) |
| **BMI** | *No limitations* | 24-40 $kg/m^2$ |
| **SU-treatment** | ≥ 5 years | no SU during the past year |
| **Other** | - | Fasting C-peptide/glucose ratio ≥ 0.045* |

** The cut-off value for fasting ratio C-peptide/glucose ≥ 0.045 as an inclusion criterion was based on data from **Study II**, in which the lower quartile of fasting ratio C-peptide/glucose in patients treated with metformin and insulin was 0.045.*

**Exclusion criteria in Studies I and III**

**Study I**: intercurrent infection or other disease that could affect metabolic control; proliferative retinopathy as assessed by an eye exam within the previous 12 months; inability to perform self monitoring of plasma glucose (SMPG).

**Study III**: intercurrent diseases of major importance e.g. severe heart disease; renal insufficiency (serum creatinine > 130 μmol/l); liver transaminases three times the normal value, untreated proliferative retinopathy; inability to perform SMPG; SU treatment during the previous year.

## Patient characteristics

Patient characteristics at baseline (**Studies I, III, IV**) or at follow-up (**Study II**) are summarized below.

**Table 3**. Summary of patient characteristics. Data are given as median and range.

| | **Study I** | **Study II** | **Study III** | **Study IV** |
|---|---|---|---|---|
| Number of patients | 25 | 171 | 43 | 14 |
| Sex (% women) | 20% | 38% | 42% | 43% |
| Age (years) | 67 (59-83) | 68 (40-87) | 66 (46-74) | 60 (53-69) |
| Diabetes duration (years) | 19 (8-29) | 14 (8-46) | 16 (10-30) | 10 (3-29) |
| *SU therapy (years)* | 15 (7-24) | *n/a* | *n/a* | 10 (1-29) |
| *Insulin therapy (years)* | 10 (6-15) | *n/a* | *n/a* | 5 (1-14) |
| BMI ($kg/m^2$) | 30 (23-44) | 30 (20-46) | 30 (25-37) | 28 (24-33) |
| Insulin dose (U/kg) | 0.50 (0.29-1.06) | - | 0.50 (0.09-2.68) | 0.52 (0.11-1.46) |
| Fasting P-glucose (mmol/l) | 8.5 (4.7-13.7 | 8.4 (2.3-20) | 8.7 (3.6-16.7) | 9.1 (4-20.6) |
| $HbA_{1c}$ (Mono-S, %) | 7.0 (5.8-8.3) | 6.7 (4.5-13) | 7.2 (5.9-8.3) | 6.9 (5.7-8.4) |
| $HbA_{1c}$ (IFCC, mmol/mol) | 63 (50-76) | 59 (36-125) | 65 (51-76) | 61 (49-77) |
| Fasting C-peptide (nmol/l) | 0.67 (0.26-1.80) | 0.70 (0.04-3.2) | 0.69 (0.09-2.1) | 0.58 (0.28-0.94) |
| Ratio fasting C-peptide/glucose | 0.083 (0.021-0.300) | 0.082 (0.003-0.457) | 0.084 (0.025-0.233) | 0.075 (0.023-0.250) |
| Postprandial C-peptide (nmol/l) | 1.70 (0.72-3.30) | 1.4 (0.04-3.8) | 1.50 (0.30-2.70) | *n/a* |
| Ratio postprandial C-peptide/glucose | 0.108 (0.038-0.265) | 0.128 (0.004-0.561) | 0.125 (0.036-0.237) | *n/a* |

Depending on study protocol and inclusion criteria, patients included in the studies had different glucose-lowering therapies as summarized in Table 4. Only a few patients were on short-acting insulin secretagogues (glinides/meglitinides) and they are therefore presented together with SU. In **Study I** all patients, by protocol, were treated with an SU compound (76% on glibenclamide, 16% on glimepiride, 8% on glipizide).

**Table 4**: Glucose-lowering therapy. Percentage of patients on different treatments.

| | **Study I** *(baseline)* | **Study II** *(baseline)* | **Study II** *(at follow-up)* | **Study III** *(baseline)* |
|---|---|---|---|---|
| Diet only | - | 17 % | 4 % | - |
| Metformin | 60 % | 37 % | 55 % | 100 % |
| SU/glinides | 100 % | 56 % | 48 % | 0 % |
| Acarbose/TZD | - | 7 % | 2 % | - |
| Insulin | 100 % | 33 % | 74% | 100 % |

In **Study I** and **Study III** patients were on various insulin regimens at baseline (Table 5) and these were not changed during the studies. The insulin regimens in **Study II** were not recorded.

**Table 5**: Percentage of patients on different insulin regimens.

| | **Study I** | **Study III** |
|---|---|---|
| ***Once-daily regimens*** | | |
| Bedtime NPH or glargine | 8 % | 23 % |
| ***B.i.d. regimens*** | | |
| Morning premixed + dinner premixed | 32 % | 23 % |
| Morning premixed + bedtime NPH | 12 % | 7 % |
| Morning + bedtime NPH/glargine/detemir | 32 % | 7 % |
| ***3-5 dose regimens*** | | |
| Morning, lunch and dinner premixed | 4 % | - |
| Morning + dinner premixed + pre-lunch analogue | 4 % | - |
| Pre-meal analogue + bedtime NPH/glargine/detemir | 8 % | 40 % |

## STUDY AIMS AND PROTOCOLS

### Study I

*Aims:* The aim of **Study I** was to assess the effect of SU in patients on long-term therapy with insulin + SU ± metformin by withdrawal of SU, and to identify clinically useful markers of long-term response.

*Study protocol:* At study start FPG, C-peptide and $HbA_{1c}$ were measured. Postprandial values for glucose and C-peptide were drawn after a standard breakfast. Before SU withdrawal FPG was rechecked at visit 2, and the mean value of FPG was defined as the patient's reference glucose value. After visit two the patients stopped their SU medication but continued all other treatments. No insulin dose adjustments were allowed. After SU withdrawal FPG and C-peptide were measured every 2-3 days and after two weeks $HbA_{1c}$ was repeated.

Patients performed SMPG daily. At two weeks SU was restarted if FPG exceeded the reference value ≥ 40% or if P-glucose during daytime was > 20 mmol/l. Patients whose glucose values did not increase according to these criteria continued without SU medication. Since 19 of 25 patients were on glibenclamide, whose glucose-lowering effect may be long-lasting [Jonsson et al. 2001], the patients whose glucose values did not increase according to the above criteria were scheduled for a follow-up visit within 8 weeks when $HbA_{1c}$ was measured. If patients' SMPG values indicated worsened glycaemic control during this time, an earlier follow-up visit was scheduled. At the follow-up, glycaemic control was evaluated with SMPG and $HbA_{1c}$, and SU was restarted if glycaemic control was assessed as impaired.

### Study II

*Aims:* The aim of **Study II** was to describe long-term changes in glycaemic control and β-cell function assessed as postprandial C-peptide and C-peptide/glucose ratio, in relation to treatment with SU over 10 years.

*Study protocol and patients:* In 1997/1998, 462 patients with type 2 diabetes attended four-day diabetes courses at the Diabetes Day Care Centre. Reasons for attending the course are shown in figure 4. Approximately ten years later all patients were invited to a follow-up visit at which point 26% of the original group had died (Fig. 4).

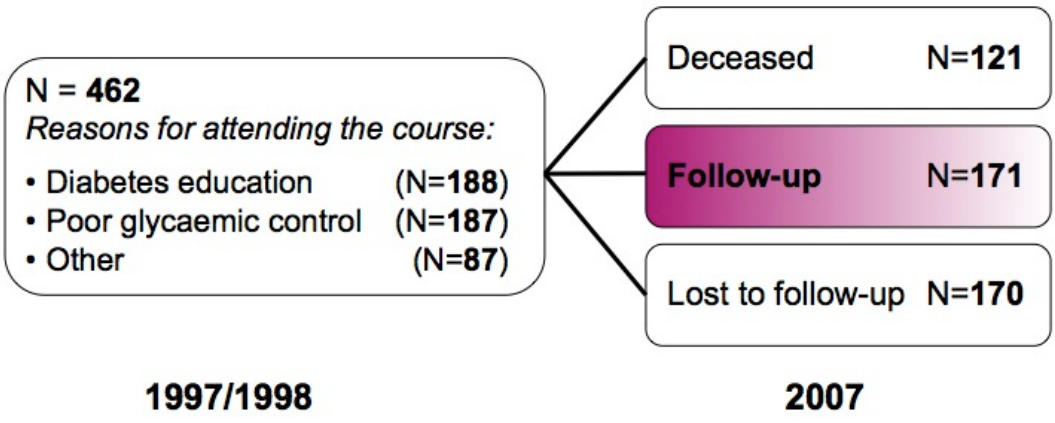


**Figure 4.** Patient flow in **Study II**.

$HbA_{1c}$, postprandial values of C-peptide and P-glucose were measured at baseline 1997/98. These measurements were repeated at follow-up, with the addition of fasting values of C-peptide and glucose and GAD antibodies. The participants filled in a questionnaire about their medical history and current diabetes treatment and, if needed, their medical records were obtained from their primary health care physician to confirm on-going and/or earlier diabetes treatment.

There were no differences in patient characteristics at baseline between the 171 patients that participated and the 170 non-participants. To further characterize the non-participants, recent $HbA_{1c}$ values and weight were obtained from 105 patients and, again, no differences were found between the groups. Glucose-lowering therapy at baseline and follow-up are shown in Table 4 and figure 8. Of the patients treated with oral glucose-lowering drugs in 1997/98, 56% were on a SU compound and in 2007 42% were on SU (6% were on meglitinides). Combined therapy with oral glucose-lowering drugs and insulin increased from 18% to 52%.

### Study III

*Aims:* The aim of **Study III** was to investigate the effect of SU in patients on metformin + insulin therapy – assessed as $HbA_{1c}$ and by CGMS – by adding glimepiride, and to identify predictors for long-term responsiveness to SU.

*Study protocol and patients:* A randomized, placebo-controlled, double-blind, cross-over study that consisted of two treatment periods of 12 weeks each, separated by a six-week washout period (Fig. 5). The patients were randomly assigned to receive Amaryl® (glimepiride) followed by placebo or the reverse sequence, on a 1:1 basis. Glimepiride dose was up-titrated during the first two weeks to a dose of 4 mg. Patients continued with the same dose of metformin as before the study and their insulin regimen was unchanged.

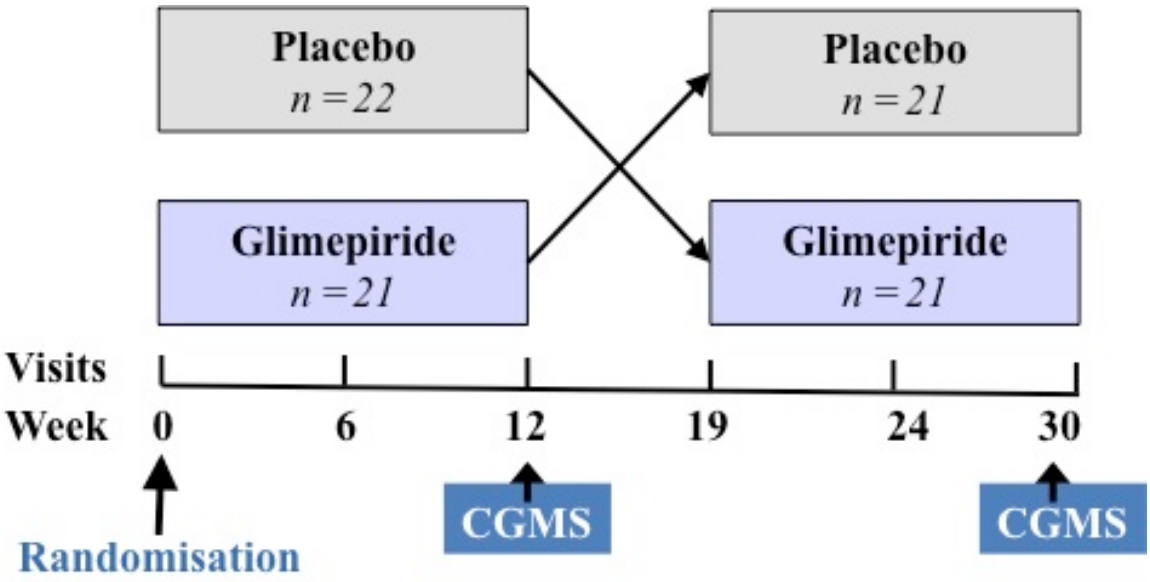


**Figure 5.** Design of **Study III.**

All the patients were asked to perform SMPG daily with additional measurements if symptoms of hypoglycaemia occurred. If fasting P-glucose was < 5.0 mmol/l or the patient had hypoglycaemia, insulin dose reduction was considered. Hypoglycaemia was defined as grade 1: only symptomatic and P-glucose > 3.1 mmol/l, grade 2 (minor): P-glucose < 3.1 mmol/l and grade 3 (major), requiring assistance from a third person [Holman et al. 2007].

Six visits were scheduled (Fig. 5) but during the first weeks of each treatment period, during up-titration of the study drug, telephone contacts with a nurse were also scheduled to assess the patient's SMPG and insulin doses for possible dose reduction. $HbA_{1c}$, fasting P-glucose and C-peptide were measured at start of each treatment period, after six weeks and at the end of treatment. Postprandial samples for glucose and C-peptide were drawn 1.5 hours after a standardized breakfast at start and end of each period. During the washout period, insulin doses, if reduced during the first treatment phase, were readjusted so as to reset to the dose at start.

During 72 hours at the end of each treatment period, glucose values were recorded with the MiniMed CGMS Gold™ (Medtronic, version 3.0, Northridge, CA). CGMS recordings were analysed according to criteria for optimal accuracy given by Medtronic. Nocturnal hypoglycaemia during CGMS was defined as a set of at least two consecutive measurements < 3.1 mmol/l between 00-06 h. Time spent at these levels was calculated.

Sixty-six patients were screened. Twenty-three were not eligible, 11 because of a low fasting ratio C-peptide/glucose (< 0.045), 11 because of $HbA_{1c}$ not being between 6.0-8.5% (Mono-S) and one because of elevated serum creatinine. Of the 43 patients that were randomized, twenty had been treated with an SU compound before; however not during the previous year since this was an exclusion criterion, with variable duration of SU therapy from a few months up to fifteen years.

## Study IV

*Aims:* **Study IV** aimed to examine the accuracy of nocturnal continuous glucose monitoring (CGMS) in patients on combined oral and insulin therapy.

*Study protocol and patients:* The CGMS was inserted into subcutaneous fat tissue of the abdomen at least 15 cm from the insertion site of the insulin pump and calibrated according to Medtronic guidelines [Gross et al. 2000]. Each patient remained ambulatory, living their ordinary life, and sensors were calibrated using four capillary plasma glucose measurements between 7 AM and 10 PM. After two or three days subjects were admitted to the Clinical Research Centre. Patients took insulin as per their current routine. From 8 PM to 6 AM, venous blood was drawn seven times for immediate analysis (Fig. 6). These samples are referred to as "reference values" and were not used for calibration of the CGMS.

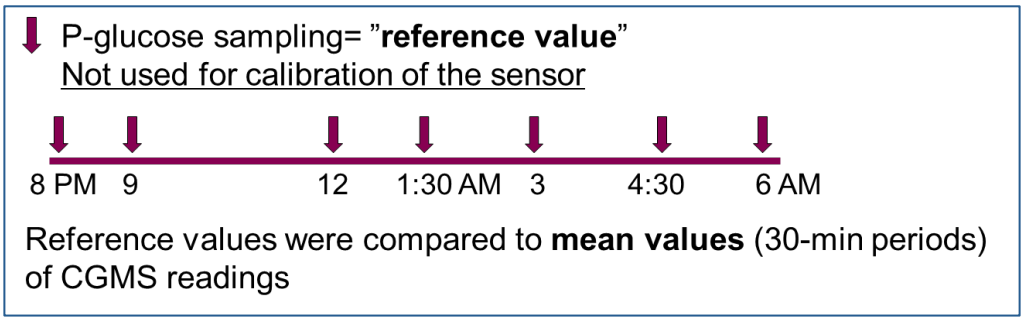


**Figure 6.** Overview of schedule for overnight sampling in **Study IV.**

The subjects were recruited among patients referred to the Diabetes Day Care Centre due to poor glycaemic control and subsequently enrolled in a clinical study to improve glucose control with insulin given as NPH insulin b.i.d. + repaglinide (NovoNorm®) or basal insulin delivered by an insulin pump (H-TronV100®) to supply a basal dose of Humalog® insulin. All patients received metformin and a fixed pre-meal dose of repaglinide of 4 mg three times daily.

## LABORATORY ANALYSES

*Plasma glucose* was analysed with a glucose oxidase method (Beckman instruments) in **Studies I-III**. In **Study IV** *plasma glucose* was analysed with Precision Plus, Medisense.

*$HbA_{1c}$* was measured using high-performance liquid chromatography (Variant II) Mono-S with a normal value of less than 5.2% and a coefficient of variation (CV) of 2.6 % in **Studies I** and **II** and of 1.55% in **Study III**.

*Serum-C-peptide* concentrations were measured immunologically using commercial kits: in **Studies I** and **II** Auto DELFIA, and in **Study III** Modular E, reference value 0.25-1.0 nmol/L and a CV of 8% and 3% respectively.

*Antibodies against Glutamic Acid Decarboxylase* (GAD) were analysed by radioimmunoassay: Diamyd Anti-GAD65 RIA in **Study I** and **IV** and enzyme immunoassay, Medizym Medipan GMBH in **Studies II** and **III** – all with a negative value ≤ 5 IE/ml.

## STATISTICS

The statistical analyses were performed using the STATISTICA software, version 7 in **Study I** and version 8 in **Studies II** and **III** (StatSoft® Inc., Tulsa, OK, USA). The JMP package 3.1.5 (SAS Institute Inc., Cary, NC, USA) was used in **Study IV**. For **Study III** the statistics were performed by a statistician using R for Statistical Computing (version 2.14.1, Vienna, Austria) for the linear mixed model and regression analyses.

*P* values < 0.05 were considered statistically significant in all the studies.

**Study I**

After validation for normal distribution using the Shapiro-Wilk test, the Student's *t*-test for paired continuous variables was used for comparisons between baseline and end values. The unpaired *t*-test was used for comparisons between the groups "responders" and "non-responders". Simple regression analysis was used to ascertain the correlation between parameters. To evaluate factors related to change in FPG a stepwise linear regression was used. Factors considered were age, diabetes duration, $HbA_{1c}$, weight, insulin dose/kg and fasting C-peptide/glucose ratio.

**Study II**

A simple linear regression analysis was used to evaluate the relation between postprandial C-peptide/glucose ratio and $HbA_{1c}$ and diabetes duration. A multivariable regression model was performed to evaluate the effects on long-term change in postprandial C-peptide/glucose. The difference in C-peptide/glucose ratio between 1997/98 and 2007 was used as outcome (dependent variable). Variables used in the regression model were gender, baseline age, BMI, duration and baseline postprandial C-peptide/glucose ratio, change in $HbA_{1c}$ (classified in categories: $< -0.2$, $> 0.2$, and -0.2 to 0.2), GAD antibody class ($\geq 200$ and $< 200$) and SU treatment (classified as continuously, periodically or never). A backward selection (exclusion criterion: $P > 0.05$) was used to decide which variables to include in the final model. Baseline BMI, duration, change in $HbA_{1c}$, SU treatment, baseline postprandial C-peptide/glucose and GAD were included regardless of *P*-value. To evaluate the effect of a single predictor on the regression, the difference in $R^2$ (coefficient of determination) between the final model and a reduced model was calculated.

**Study III**

The primary endpoint $HbA_{1c}$ was analysed using a linear mixed model where $HbA_{1c}$ at visits 2 and 3 within each period were included as the dependent variable. Group, time, baseline $HbA_{1c}$ in each period, sequence and period were added as fixed factors, and subject was added as a random factor. Based on this model the difference between treatment groups can be inferred. The interaction between group and time was evaluated and considered non-significant. To evaluate factors related to a change in $HbA_{1c}$ within the treatment group a stepwise linear mixed model was used. Factors considered were age, diabetes duration, baseline values of weight, $HbA_{1c}$, insulin dose and the difference between fasting and postprandial C-peptide/glucose ratio (increment). To evaluate factors related to hypoglycaemia a stepwise logistic regression model was performed. The dependent variable was the occurrence of hypoglycaemia and the factors considered were age, diabetes duration, change in $HbA_{1c}$, weight, insulin dose/kg and fasting C-peptide/glucose ratio. A power calculation was carried out. According to this, to detect a difference in $HbA_{1c}$ of 0.8% between glimepiride/placebo treatment period, with an $\alpha$ error of 0.05 and a statistical power of 80%, a sample size of 41 patients was needed.

**Study IV**

Pearson´s correlation coefficients between reference glucose values and calculated mean CGMS readings were calculated. To assess agreement between the CGMS and reference method, Bland and Altman plotting [Bland et al. 1986] was used. An Error grid analysis was performed according to a new, modified consensus Error grid from 2000 [Parkes et al. 2000] which is based on the original Error grid [Clarke et al. 1987].

# RESULTS

## GLYCAEMIC CONTROL

### Glycaemic control in Study II

Ten years after they had attended a diabetes course at the Diabetes Day Care Centre, the patients' $HbA_{1c}$ had decreased (Fig. 7) from 7.41% to 6.96% (mean difference: -0.45; 95% CI: -0.73, -0.16; $P = 0.002$). Expressed in IFCC units, $HbA_{1c}$ had decreased from 67 to 62 mmol/mol.

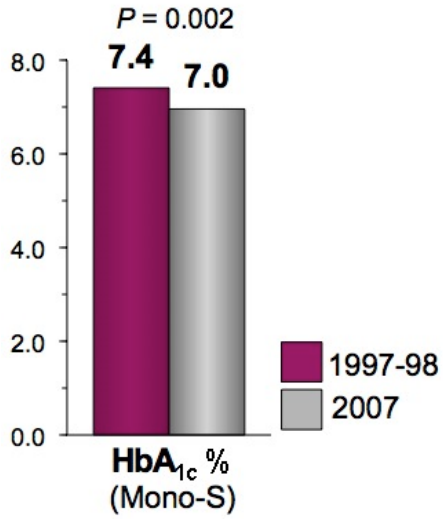


**Figure 7.** Change in $HbA_{1c}$

This improvement in glycaemic control despite a longer duration of diabetes had been achieved through an intensification of glucose-lowering treatment (Table 4 and Fig. 8), e.g. increased insulin use: at follow-up 74% were on insulin, alone or in combination with oral medication, compared to only 33% at baseline.

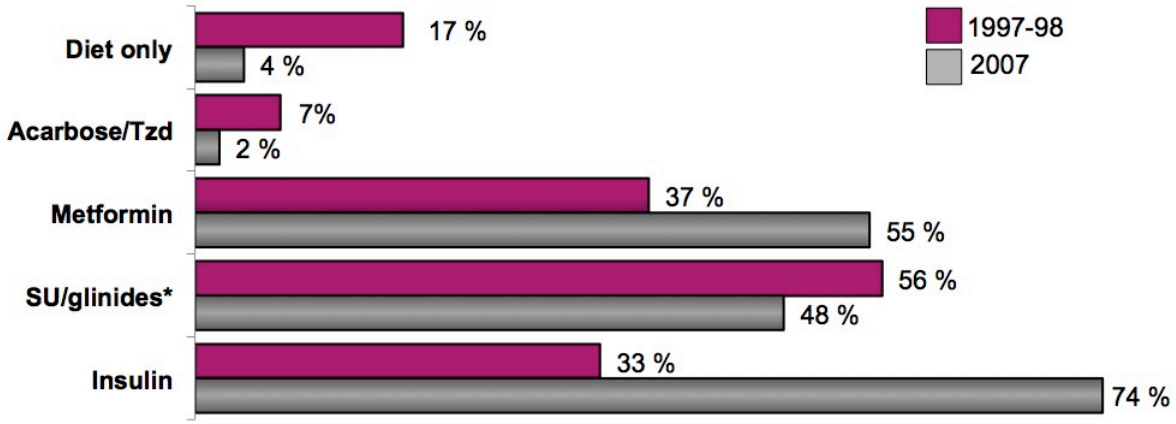


**Figure 8.** Use of glucose lowering treatments in 1997/1998 and 2007.

### Glycaemic effects of SU in Study I

After withdrawal of SU, FPG increased by ≥ 40% in 4/25 patients and another four reported an increase in plasma glucose > 20 mmol/l during daytime. Together these eight subjects were referred to as "early SU restarters" (Fig. 9). At follow-up in median 6 weeks (range 4-8) after SU withdrawal, another twelve patients were restarted on SU because of deterioration in glycaemic control, and they thus were referred to as "late SU restarters" (Fig. 9). Considering early and late restarters together, 80% of the patients could be classified as SU responders.


**Figure 9.** Classification of patients after SU withdrawal in **Study I.**

$HbA_{1c}$ among the "late restarters" increased from 7.1% to 8.2% (64-75 mmol/mol; $P < 0.001$) at follow-up (Fig. 10), the median increase being 1.1%, (range 0.4-2.0%; IFCC 4-21 mmol/mol).

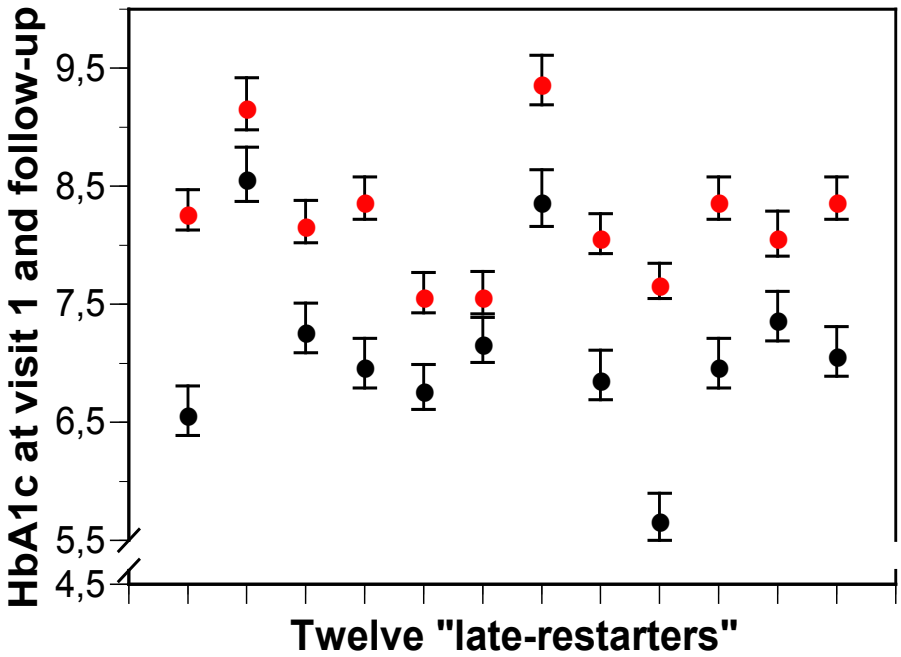


**Figure 10.** $HbA_{1c}$ before SU withdrawal (●) and at follow-up visit (●) in each "late restarter". The bars represent the CV for the analysis.

### Glycaemic effects of SU in Study III

$HbA_{1c}$ decreased from 7.0% to 6.4%, (63-56 mmol/mol; $P < 0.001$) three months after addition of glimepiride to insulin and metformin. The median decrease was 0.5% (5 mmol/mol) and ranged from -0.1% to 1.9% (1-20 mmol/mol), FPG decreased from 8.6 to 7.3 mmol/l ($P < 0.01$), (Fig. 11 and 12). There was no change in $HbA_{1c}$ during placebo treatment.

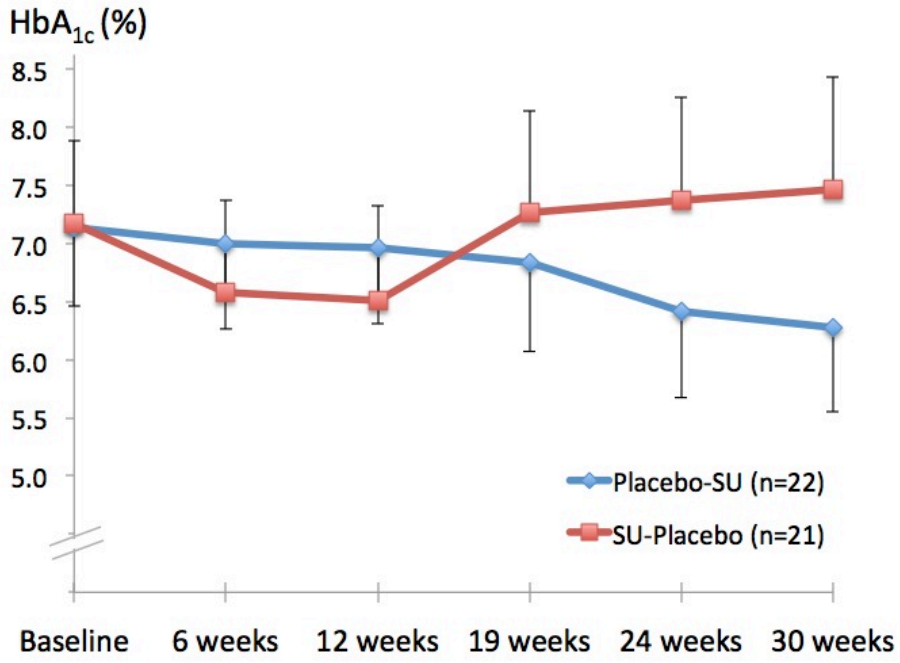


**Figure 11.** $HbA_{1c}$ (mean ± SD) presented according to treatment sequence**.**

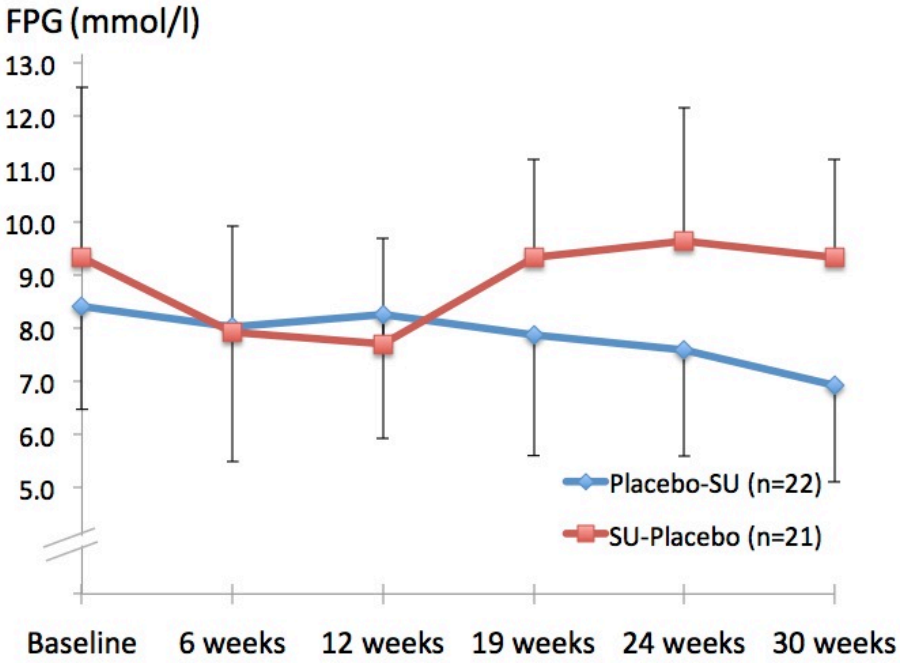


**Figure 12.** FPG (mean ± SD) presented according to treatment sequence**.**

The improvement in $HbA_{1c}$ after glimepiride addition was accompanied by a need to reduce insulin according to protocol to avoid hypoglycaemia. The insulin doses thus had to be reduced in 23/43 of the patients by 2-100% (median 29%). In this subgroup, $HbA_{1c}$ decreased in median by 0.6%. When comparing patients who had to reduce their insulin dose with those who did not, $HbA_{1c}$ at start of glimepiride was 6.7% vs. 7.4% (59 vs. 67 mmol/mol; $P = 0.001$). There was no relation between insulin dose/kg and insulin dose reduction.

There were no differences in baseline variables between treatment sequence groups in this cross-over study, except in FPG (9.3 vs. 8.4 mmol/l, $P = 0.028$), see figure 12; but $HbA_{1c}$ was similar between groups (7.1 vs. 7.2%, IFCC 64 vs. 65 mmol/mol; $P = 0.855$), see figure 11. Further, there was no effect in randomization sequence or in $HbA_{1c}$ (7.0% vs. 7.2%, 63 vs. 65 mmol/mol; $P = 0.106$) at the start of the two treatment periods. This was because patients starting with glimepiride and whose glycaemic control improved during this treatment period deteriorated in $HbA_{1c}$ during the washout period.

## β-CELL FUNCTION AND PREDICTION OF RESPONSE TO SU

### Study I

When comparing patients classified as SU responders (N=20) with the non-responders (N=5), the responders had higher levels of basal fasting C-peptide (0.84 ± 0.44 vs. 0.41 ± 0.15 nmol/l; $P < 0.05$), with a large variation in the group. No other differences were observed between the groups at baseline ($HbA_{1c}$, FPG, fasting or postprandial C-peptide/glucose ratio or increase in C-peptide from fasting to postprandial, BMI or insulin requirement).

In a prediction analysis the parameters associated with an increase in FPG when SU was withdrawn was diabetes duration ($P < 0.01$) and insulin dose/kg ($P = 0.02$). There was a positive relation between the change in FPG (from baseline to the two-week visit) and diabetes duration ($P < 0.01$) as well as between the change and duration of SU treatment ($P < 0.001$). Patients with a longer duration had a more pronounced increase in FPG (Fig. 13), indicating that they had had more benefit from SU than those with a shorter duration. The insulin dose/kg correlated inversely with the increase in FPG i.e. patients needing a higher insulin dose did not seem to benefit from SU as much as those with a lower dose.

The different insulin regimens (Table 5) or the use of metformin did not affect the changes observed in FPG and $HbA_{1c}$ following withdrawal of SU.

In the SU responders, fasting C-peptide levels decreased from 0.84 to 0.53 nmol/l ($P < 0.001$) after SU withdrawal, as compared with the non-responders in whom the concentration did not change significantly (0.42 vs. 0.41). The same was observed for the ratio C-peptide/glucose, which decreased significantly after SU withdrawal: in the fasting state from 0.104 to 0.050 ($P < 0.001$) and postprandially from 0.130 to 0.076 ($P < 0.001$).

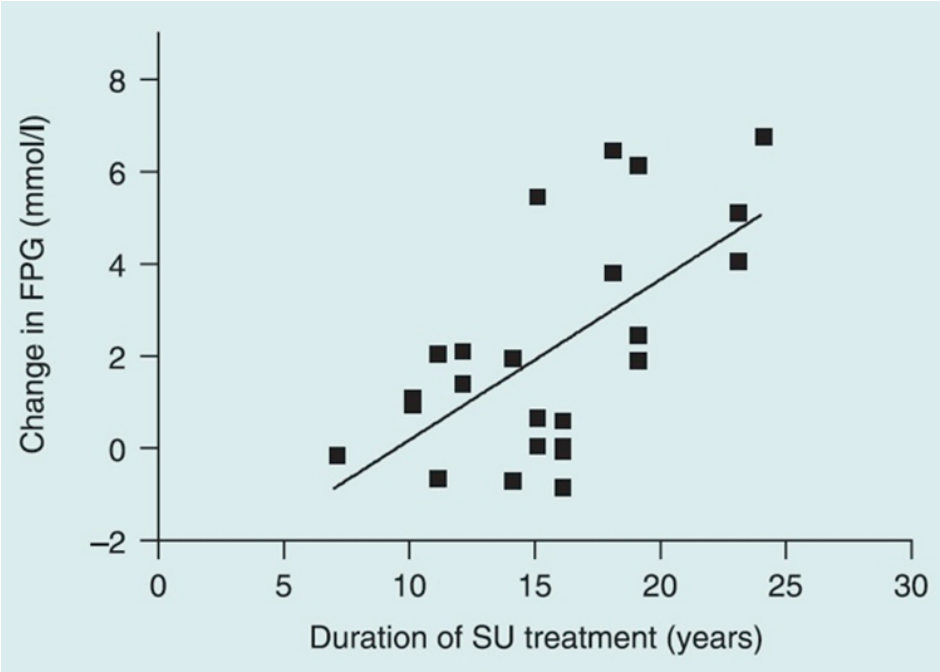


**Figure 13.** Relation between change in FPG from study start to end of two week withdrawal and duration of SU therapy ($R^2 = 0.26$, $P < 0.01$).

## Study II

After ten years there was a decrease in postprandial C-peptide and postprandial C-peptide/glucose ratio, as shown in figure 14.

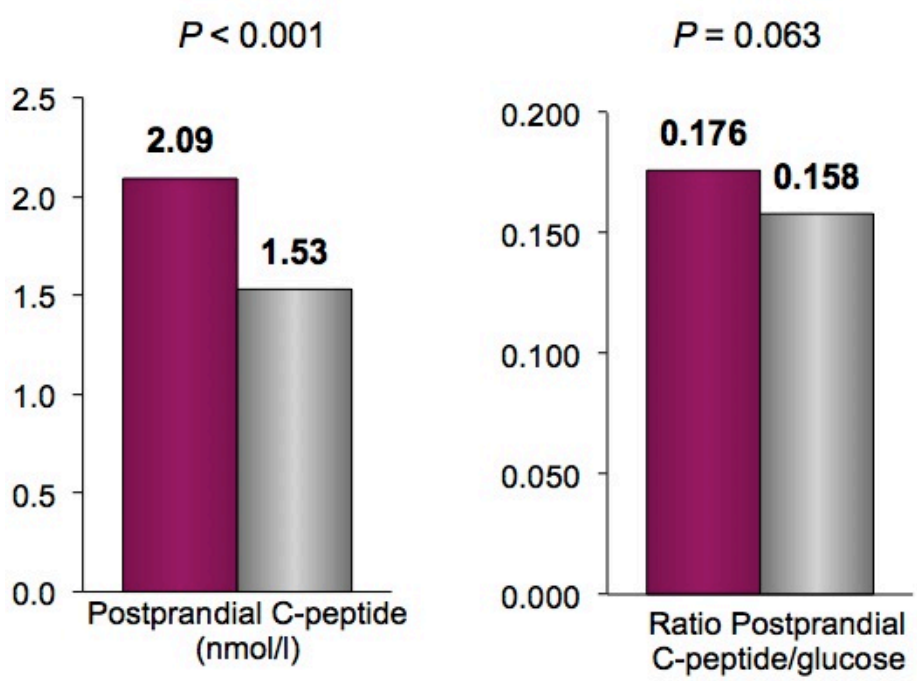


**Figure 14.** Change in postprandial C-peptide and C-peptide/glucose ratio

A positive titre (> 200 U/ml) of GAD antibodies was found in twelve patients (7%). The GAD-positive patients differed from the GAD-negative in BMI, postprandial C-peptide and post-prandial C-peptide/glucose ratio and their ratio decreased from 0.137 to 0.081 (mean difference: -0.056, 95% CI: [-0.11; 0.002], $P = 0.056$). If the twelve GAD-positive patients were excluded from the analyses, the decrease in the ratio was lower and non-significant ($P = 0.139$), but other parameters analysed were unaffected.

All C-peptide measurements were negatively related to diabetes duration. In a simple linear regression model the strongest correlation was with postprandial values (Fig. 15).

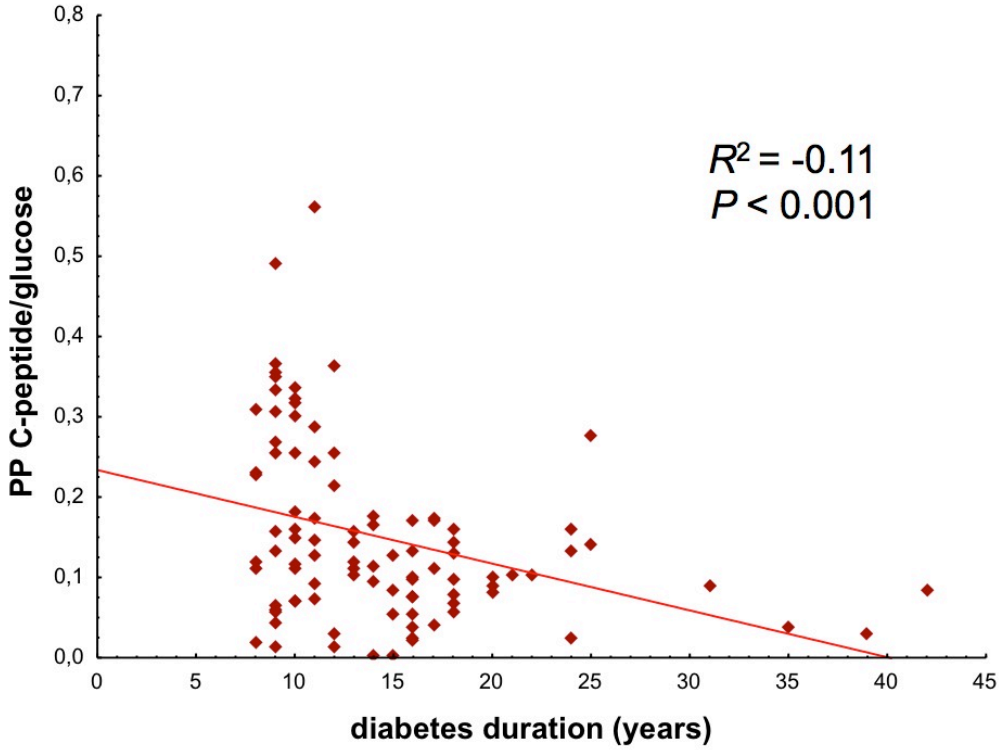


**Figure 15.** Correlation between postprandial C-peptide/glucose ratio and diabetes duration

A multiple regression model was performed to evaluate the effects on long-term change in post-prandial C-peptide/glucose. This regression model revealed that the baseline postprandial C-peptide/glucose ratio was the most important independent variable ($R^2 = 45\%$; $P < 0.001$) for explaining the variation in long-term changes in the ratio. The second most important variable was insulin therapy ($R^2 = 7\%$; $P < 0.001$), while change in $HbA_{1c}$ from baseline to follow-up was the third most important variable ($R^2 = 5\%$; $P < 0.001$). An increase in $HbA_{1c}$ between 1997/1998 and 2007 was associated with a decrease in postprandial C-peptide/glucose ratio (mean change: -0.056). The ratio remained essentially unchanged when $HbA_{1c}$ was decreased. The model explained 61% of the variance in long-term ratio C-peptide/glucose.

*Long-term exposure to SU and β-cell function*

To assess a possible effect of SU treatment on β-cell function, patients in **Study II** were classified into three groups depending on SU exposure: continuously during ten years, periodically or never. These groups were used in the multiple regression analysis with the difference in ratio post-prandial C-peptide/glucose between 1997/98 and 2007 as the outcome variable. Treatment with SU could not explain any of the observed variation in long-term change in postprandial C-peptide/glucose ($R^2 = 0.1\%$, $P = 0.89$).

### Study III

The ratio between C-peptide/glucose did not change during the placebo period but increased significantly with glimepiride, in the fasting state from 0.087 to 0.118 ($P < 0.001$) and post-prandially from 0.123 to 0.173 ($P < 0.001$).

If response to glimepiride was defined as a decrease in $HbA_{1c}$ of ≥ 0.5% (5 mmol/mol) or a reduction in total insulin dose of ≥ 20%, 29/43 patients (67%) met these criteria and they were classified as responders. When comparing the responders (N=29) to the non-responders (N=13), the responders were younger (64 vs. 68 years, $P < 0.05$) but no other differences were observed between the groups.

To analyse factors at baseline that could be of value to predict response to glimepiride, a step-wise multiple regression analysis was performed with the change in $HbA_{1c}$ as dependent variable. This analysis revealed that a larger increment i.e. the increase from fasting to post-prandial values, in C-peptide/glucose ratio was associated with a more pronounced decrease in $HbA_{1c}$ ($P < 0.001$). Older age was associated with a smaller decrease ($P < 0.05$). These two variables together explained 26% of the variance in $HbA_{1c}$.

## HYPOGLYCAEMIAS

In **Studies I**, **II** and **IV** hypoglycaemia was not defined by protocol, and thus hypoglycaemia could only be correctly assessed in **Study III**.

### Hypoglycaemia by SMPG

Patients performed SMPG every morning but also if symptoms of hypoglycaemia occurred. Minor hypoglycaemia, confirmed by SMPG, occurred in 22 patients. They reported a total of 124 episodes during the 3+3 months, with the majority of these (74%) occurring during the glimepiride period. With glimepiride 21 patients reported altogether 92 episodes (1-8 episodes/per patient, except one subject who experienced 30 episodes) with a SMPG < 3.1 mmol/l.

A stepwise logistic regression model revealed that variables relating significantly to hypo-glycaemia in the glimepiride group was age and baseline fasting C-peptide/glucose ratio so that an increase in age by one year increased the odds of having an event by about 21 % (Odds ratio 1.21, CI: 1.05-1.49, $P = 0.03$) and an increase in fasting C-peptide/glucose by 0.01 decreased the odds with 31% (Odds ratio 0.69, CI: 0.48-0.89, $P = 0.015$).

### Nocturnal hypoglycaemia by CGMS

Glycaemia was monitored by CGMS for 72 hours at the end of each treatment period. CGMS monitoring could not be performed in 4 patients. When evaluating CGMS recordings between midnight and 6 AM, 4% of the recordings did not fulfil accuracy criteria and were not analysed. Six patients (15%) had two or more consecutive values < 3.1 mmol/l during the last three nights with placebo, the median time spent at these levels with placebo was 40 min (range 5-105). At the end of glimepiride treatment 15 patients (38%) had two or more consecutive values < 3.1 mmol/l and the median time spent at low glucose levels was 45 min (5-280). Notably, none of these recordings were associated with symptoms.

## ACCURACY OF CGMS

All CGMS data were evaluated according to the quality criteria for optimal accuracy given by Medtronic [Gross et al. 2000] and 32 % of the data were excluded from further analysis. One hundred and three paired CGMS and glucose reference values from 12 patients were available, 40% after 48 hours and 60% after 72 hours. A correlation coefficient of 0.80 ($P < 0.001$) was seen after 48 hours and a lower coefficient of 0.33 ($P = 0.01$) after 72 hours. Bland-Altman analyses [Bland et al. 1986] demonstrated that the dispersion of the values, expressed as mean ± 2SD, around the mean of differences was wider after 72 hours than after 48 hours.

Plotting CGMS and glucose values according to the Clarke Error Grid analysis, which is used to assess the accuracy of a given glucose method with respect to the clinical decision process [Parkes et al. 2000], demonstrated that 100 % of data were within zones A and B after 48 hours with 60 % in zone A (Fig. 16, left panel). However, only 44 % were in zone A after 72 hours, in parallel with which 7 % of data were “clinically unacceptable” i.e. in zone D (Fig. 16, right panel).

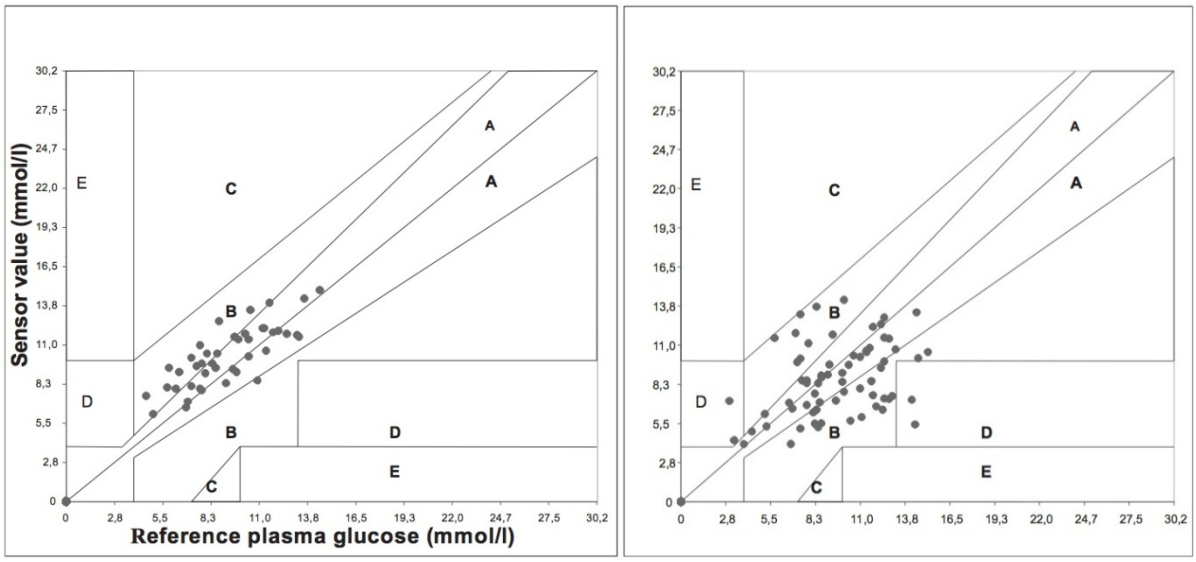


**Figure 16.** Clarke Error grid analysis comparing CGMS values to P-glucose after 48 hours (left panel) and 72 hours (right panel).

# DISCUSSION

## ASSESSMENT OF INSULIN SECRETION IN CLINICAL PRACTICE – IS IT USEFUL?

In clinical practice, tests for assessing β-cell function, i.e. insulin secretion, must be easy to perform. For this reason, fasting samples or simple stimulation tests have to be used. HOMA-B, using fasting insulin and glucose, is the most validated method and has been used in large clinical studies [UKPDS 1998; Kahn et al. 2006]. However it cannot be used in patients with insulin therapy and is thus mostly not applicable in patients with longer diabetes duration because it is these who receive insulin therapy.

A commonly used parameter for evaluating endogenous insulin secretion is C-peptide but the interpretation of C-peptide values can be very difficult - because of the many factors influencing insulin secretion, which include prevailing glucose level, chronic glucotoxic effects of elevated glucose levels, and treatment with hypoglycaemic agents [Albareda et al. 2005]. C-peptide response to a stimulus is affected by the pre-stimulatory glucose level, and if glucose is acutely elevated in well-controlled type 2 patients, C-peptide response is enhanced [Gjessing et al. 1989] but when chronic hyperglycaemia and glucotoxicity exist, the insulin secretory response may be blunted [McFarlane et al. 2001; Buchanan 2003].

Since glucose is a major stimulant of C-peptide secretion [Brunzell et al. 1976], C-peptide values should be interpreted in the context of the prevailing glucose levels; and one approach to this is via the C-peptide/glucose ratio. Treatment with exogenous insulin is associated with a decrease in C-peptide concentrations, a finding that is probably related to lower glucose concentrations [Lindström et al. 1992]. This was also found in **Study II,** in which C-peptide levels decreased markedly over ten years in patients on insulin therapy. However, the C-peptide/glucose ratio remains unchanged during insulin therapy [Lindström et al. 1992; Albareda et al. 2005], which is in line with what was observed in **Study II**, where the ratio decreased only slightly in the insulin-treated patients.

An experimental study by Meier et al, recruiting patients who were to undergo pancreatic surgery, tried to assess what measure of insulin secretion showed the closest association with β-cell area [Meier et al. 2009]. Patients were given an oral glucose load and different measures of insulin secretion were obtained during a prolonged OGTT and β-cell mass was later collected during surgery. It was concluded that the C-peptide/glucose ratio showed the best correlation, while HOMA-B failed to predict β-cell area [Meier et al. 2009]. It was suggested that future studies should consider using the C-peptide/glucose ratio after oral glucose ingestion when evaluating β-cell function over-time.

In **Study II** long-term β-cell function was assessed as postprandial C-peptide/glucose ratio. Since no fasting values were obtained at baseline in 1997/98 it was not possible to calculate β-cell responsiveness at baseline or to use these measurements for evaluation of long-term β-cell function. Evaluating the relation between different C-peptide measurements with diabetes

duration and $HbA_{1c}$ at follow-up in **Study II** showed that postprandial C- peptide values related more closely to both diabetes duration and $HbA_{1c}$ than fasting values did. This was also the finding in another clinical study in which β-cell function was evaluated by using C-peptide [Haupt et al. 1999].

A meaningful assessment of C-peptide response to a stimulus such as a meal includes a consideration of the baseline C-peptide levels. When developing the indexes based on mathematical modelling for fasting ratio C-peptide/glucose and postprandial β-cell responsiveness (ΔC-peptide/Δglucose) Hovorka et al evaluated a simplified method using one or two point measurements of C-peptide and glucose values. They concluded that this easier approach could be used for assessing post-prandial insulin secretion [Hovorka et al. 2001]. Postprandial responsiveness is more closely related to diabetes duration and glycaemic control than fasting values are, as reported in other studies [Albarrak et al. 2002; Shim et al. 2006] in which β-cell function is evaluated by using the increment of C-peptide/glucose.

To summarize, when evaluating insulin secretion by using C-peptide in patients with different glucose levels and glycaemic control, treated with different combinations of glucose-lowering drugs and insulin regimens, the use of the C-peptide/glucose ratio, reduces the influence of some of the confounders affecting the measurement. It seems that, although far from perfect, the ratio - especially postprandially - is more appropriate for evaluating insulin secretion in patients with type 2 diabetes. These measurements can be useful when β-cell function is being evaluated longitudinally in clinical studies. In clinical practice very low or high level of C-peptide can be helpful, mainly in the classification of diabetes, but only if interpreted in the context of prevailing glucose level and if the influence of long-term glycaemic control is considered.

## LOSS OF INSULIN SECRETORY CAPACITY IN TYPE 2 DIABETES – IS IT INEVITABLE?

The reported loss of β-cell function, as assessed by HOMA-B, that was observed over time in the UKPDS has substantially affected the general view of type 2 diabetes as a disease characterized by a deterioration of β-cell function with time [UKPDS 1995]. However, the aims of the blood glucose control in the UKPDS were twofold: (1) to test the hypothesis that tight glucose control leads to a reduction in diabetes complications and (2) to evaluate whether there are any specific advantages or disadvantages with the three commonly used glucose-lowering therapies i.e. SU, insulin and metformin. Therefore, by protocol, the patients were kept on monotherapy with their allocated treatment even though a steady increase in fasting glucose was observed which in turn, not surprisingly, led to deterioration of glycaemic control as assessed with $HbA_{1c}$. In the UKPDS, β-cell function was assessed as HOMA-B and it should be noted that the mathematical formula is such that HOMA-B decreases as FPG increases, unless there is a simultaneous increase in insulin concentration. As discussed earlier, the relationship between FPG and insulin secretion is complex and HOMA-B may not provide a "totally accurate view of the absolute ability of the pancreatic β-cell to secrete insulin" [Reaven 2009].

In **Study II** changes in β-cell function, as assessed by postprandial C-peptide alone as well as by the C-peptide/glucose ratio, were described at baseline and after ten years. One important finding was that even though postprandial C-peptide was significantly lower after ten years, the ratio was only marginally and non-significantly lower. Further, considerable variation between patients was observed.

In contrast to the UKPDS findings the patients in **Study II** showed improved glycaemic control over ten years, most likely due to an increased and intensified use of glucose lowering therapies. The importance of considering glycaemic control when interpreting changes in β-cell function, assessed by using C-peptide values as in **Study II**, is highlighted by the finding that when $HbA_{1c}$ increased C-peptide/glucose decreased and if glycaemic control improved, the ratio remained unchanged. A longer diabetes duration at follow-up was associated with lower C-peptide and lower ratio C-peptide/glucose values, and thus the results are consistent with those in other cross-sectional studies [Clauson et al. 1994; Haupt et al. 1999; Shim et al. 2006; Funakoshi et al. 2008]. Zangeneh et al reported that insulin secretion as measured by C-peptide declined with increasing diabetes duration in approximately 50% of patients, but increased or remained constant in the other half in a 12 year follow-up study in which glycaemic control was improved and GAD antibody-positive patients were excluded [Zangeneh et al. 2006]. The results are in concordance with those in **Study II**, since we observed similar patterns in the change in insulin secretion. In a 20 year prospective study of consecutively diagnosed diabetes patients in Sweden [Ekholm et al. 2012], insulin secretion, assessed as fasting C-peptide was preserved in GAD antibody negative patients, when glycaemic control was improved at the same time as a result of more intensive treatments. In **Study II** 12 patients (7%) tested GAD-antibody-positive, a prevalence similar to that observed in older patients in the Finnish Botnia study [Tuomi et al. 1999]. GAD-antibody-positivity in **Study II** was associated with lower C-peptide/glucose ratios and also with a more pronounced decrease in C-peptide/glucose after ten years. Further, when we excluded GAD-antibody-negative patients from the analyses, the ratio did not decrease over ten years.

Deterioration of β-cell function does not seem to be completely irreversible. In a study where four weeks of intensive treatment with insulin was given to a group of patients with hyperglycaemia, some with diabetes duration of 12 years, there was marked increase insulin secretion which persisted for at least two weeks after insulin treatment was stopped [Andrews et al. 1984]. However, there was "considerable patient-to-patient variation in the degree to which insulin action was enhanced" [Andrews et al. 1984]. The positive effects on glycaemic control, observed after weight reduction surgery, with remission of type 2 diabetes, in some patients for up to two years or more, also support the notion that deterioration of β-cell function in type 2 diabetes is not irreversible [Buchwald et al. 2009].

To summarize, it seems that the decline in insulin secretory capacity is variable, related to glycaemic control and is not inevitable in all patients.

## SU IN PATIENTS WITH A LONG DURATION OF DIABETES – IS IT WORTHWHILE?

An implication of the heterogeneity regarding loss of β-cell function with preserved insulin secretion in many patients is that SU treatment could be effective even after a long duration of diabetes. This issue has not been specifically addressed in earlier clinical studies.

In most studies on combination therapy with SU and insulin, diabetes duration has varied substantially, and duration has not been specified as an inclusion criterion. An Australian study concluded that patients with a duration of insulin therapy > 8 years are unlikely to benefit from adding SU to insulin [Lewitt et al. 1989]. This opinion, i.e. that SU not is advantageous in patients with a longer duration of type 2 diabetes, seems to be shared by others [Massi-Benedetti et al. 2008; Raskin 2008]. It is true that SU therapy often fails to maintain glycaemic levels when used as monotherapy, termed "SU failure" [Matthews et al. 1998]. Further, older studies investigating the efficacy of combined SU + insulin treatment have been short-term, and the long-term efficacy of SU in combination therapy has not been clarified. However, two large prospective studies have shown durability of combined SU and insulin therapy [Wright et al. 2002; Patel et al. 2008]. In a sub-study of the UKPDS [Wright et al. 2002], using a modified protocol, it was allowed to add insulin in patients allocated to SU if maximal doses of SU did not maintain FPG < 6.0 mmol/l. After six years 52% of the patients required combination therapy, and SU + insulin resulted in a lower $HbA_{1c}$ (6.6% vs. 7.1%; 49 vs. 54 mmol/mol) than that in the group treated with insulin monotherapy. The ADVANCE study [Patel et al. 2008] used gliclazide (modified release), a SU compound, as the basis for intensive glucose-lowering treatment adding other drugs as required to achieve a goal of $HbA_{1c}$ < 6.5% (48 mmol/mol). The patients included had a mean duration of diabetes of around eight years at baseline and at the end of follow-up, after a median of five years, almost 74% had metformin added to SU and insulin had been added in 40.5%, reaching an $HbA_{1c}$ of median 6.4% (46 mmol/mol). While the primary outcome in the ADVANCE study was not the efficacy of SU therapy, the results indicate that glycaemic control with combined SU and insulin can be maintained. The ability of SU to maintain its positive effect on glucose control in patients with a longer duration of diabetes was investigated in **Study I** and **Study III**.

In **Study I** - which recruited patients with a median diabetes duration of 19 years and at least five years of SU treatment - withdrawal of SU clearly resulted in a worsening of glycaemia in two thirds of the 25 patients. SU withdrawal is a procedure used in earlier diabetes studies [Nobels et al. 1989; Lev-Ran et al. 1998; Landstedt-Hallin et al. 1999]. Withdrawal studies can be designed either as a randomized study where patients are randomized to either stop or continue using the medication [Landstedt-Hallin et al. 1999; Iyer et al. 2008] or, as in **Study I**, where all patients stop SU and the effect on glycaemia is observed for each individual patient. In the two earlier SU withdrawal studies by Lev-Ran et al and Landstedt-Hallin et al, in patients with diabetes duration of mean 13.5 and 9.9 years respectively, SU withdrawal resulted in an increase in FPG of approximately 40% in 80% of patients after a follow-up of four and 17 weeks respectively. In **Study I** the follow-up was only two weeks, at which time FPG had increased by over 40% in 4/25 patients, less than in the two early-mentioned studies. However, the observation time of two weeks in **Study I** was most likely too short for metabolic deterioration to occur and moreover, the

majority of patients in **Study I** were treated with glibenclamide, which is eliminated slowly [Jonsson et al. 1994] and may have long-lasting active metabolites [Jonsson et al. 2001].

More recently the efficacy of SU in combination with insulin in long-standing diabetes was evaluated in a Japanese cross-over, withdrawal study [Yokoyama et al. 2010] of patients with a mean diabetes duration of 17 years and good glycaemic control, on treatment with combined metformin, glimepiride and insulin. The patients had been on SU treatment for ten years with the addition of insulin the previous five, very similar to the patients in **Study I**. Three months after withdrawal, glycaemic control deteriorated significantly in the discontinuation arm, from 7.0% to 8.1% (NGSP values calculated from JDS; IFCC 53 to 65 mmol/mol) i.e. $HbA_{1c}$ increased by 1.1% (12 mmol/mol) which is identical with the result in **Study I**, in which $HbA_{1c}$ increased in median 1.1% in the "late-restarters" after SU withdrawal. The authors concluded that the efficacy of glimepiride did not decline severely after more than ten years of diabetes and moreover, when they stratified subjects according to diabetes duration, patients with a duration > 17 years experienced similar worsening of $HbA_{1c}$ when SU was withdrawn [Yokoyama et al. 2010].

In **Study III** glimepiride was added to on-going insulin and metformin in patients with a known diabetes duration ≥ 10 years. The results showed significant reduction in $HbA_{1c}$ three months after glimepiride addition even though insulin doses had to be reduced in approximately 50 % of the patients. Adding SU to insulin therapy was investigated in another placebo-controlled cross-over study, where the patients had a mean diabetes duration of 15 years and an insulin requirement of ≥ 40 U/day [Feinglos et al. 1998]. Glipizide addition resulted in a rapid and substantial improvement in glycaemic control despite significant insulin dose reductions in the insulin + glipizide group. In a small, observational study the efficacy of adding gliclazide in patients in routine diabetes care, with poor glycaemic control ($HbA_{1c}$ 9%; 75 mmol/mol) and treated with different insulin regimens and metformin, mean diabetes duration of 13 years, resulted in a reduction in $HbA_{1c}$ of 1.4% (15 mmol/mol) after 3 months [Brown 2006]. In a Japanese study glimepiride was added to insulin in patients with a mean diabetes duration of 19 years and poor glycaemic control (JDS $HbA_{1c}$ 8.4%; 74 mmol/mol) which resulted in a decrease in $HbA_{1c}$ of 1.1% (12 mmol/mol) after 12 weeks, however, without significant insulin dose reductions during this period [Ose et al. 2005]. The patients were followed for 18 months and the improvement in $HbA_{1c}$ was sustained during this period while insulin doses were significantly reduced after three months in the group where glimepiride had been added [Ose et al. 2005]. In **Study III** $HbA_{1c}$ decreased in median 0.5% (IFCC 5 mmol/mol) during three months treatment with glimepiride and the insulin dose had to be reduced in 23 patients, by 2-100 % (median 29 %) as an effect of glimepiride stimulating endogenous insulin secretion. Comparing the results in **Study III** with those of Ose et al, it is notable, that baseline $HbA_{1c}$ was higher in the Japanese study, which allows for a greater reduction in $HbA_{1c}$ after SU addition. In **Study III** patients who had to reduce their insulin dose after addition of glimepiride because of low glucose values and/or hypoglycaemia had a significantly lower $HbA_{1c}$ at baseline, 6.7% vs. 7.4%, (59 vs. 67 mmol/mol). One third of the patients had a baseline $HbA_{1c}$ of 6.5% or lower (57 mmol/mol) and therefore no substantial reduction in $HbA_{1c}$ could be expected. This subgroup had a median decrease in $HbA_{1c}$ of 0.3% together with an insulin dose reduction of 30%, compared to those with a baseline $HbA_{1c}$ > 6.5% in which $HbA_{1c}$ decreased by 0.6% and the insulin dose was reduced to a lesser extent.

One could argue that a reduction in $HbA_{1c}$ of 0.5%, the median reduction achieved in **Study III**, is rather small and not clinically important. However, epidemiological data from UKPDS suggest that a reduction of $HbA_{1c}$ of 0.5% might equate to 11.5% reduction of diabetes complications [Stratton et al. 2000].

In summary, since SU in long-term combination therapy with insulin and metformin appears to be effective in many patients there is no rationale for routinely withdrawing the SU component after many years of combination therapy. However, a period of SU withdrawal, to determine whether the drug is still beneficial, can easily be performed and if glycaemia worsens it is worthwhile to recommence the medication. Furthermore, in patients with type 2 diabetes treated with metformin and insulin, addition of glimepiride to on-going therapy if the individualized glycaemic target is not reached may be worthwhile, even after many years of diabetes.

## WHAT ARE THE POSSIBLE DISADVANTAGES OF ADDING SULPHONYLUREA TO INSULIN AND METFORMIN?

The well-known disadvantages of SU are weight gain and hypoglycaemia. In the present studies no effect on weight was observed. In **Study III**, glycaemic control improved which can be associated with weight gain, but insulin doses were reduced simultaneously, and this may have promoted weight stability. Hypoglycaemic events were evaluated in **Study III** and this will be discussed below.

Another potential disadvantage of SU treatment was extensively debated during the 1990's when the mechanism of action of SU was described i.e. the binding to ATP-sensitive potassium channels that also are found in the myocardium [Ashcroft et al. 1992]. Since these channels are believed to be important for what is termed ischaemic preconditioning of the heart, partly protecting the heart during ischaemic events, blocking of them might be disadvantageous in some situations [Engler et al. 1996]. During the past decade this debate has faded and the clinical relevance of these molecular mechanisms can be questioned, especially since long-term data from the UKPDS also showed a protective effect with regards to myocardial infarction and cardiovascular death also in the group treated intensively with SU [Holman et al. 2008].

### Increased risk of hypoglycaemia? The use of CGMS for assessment?'

One negative effect of intensive glycaemic control in patients with diabetes is the increased rate of hypoglycaemia [1993; Pedersen-Bjergaard et al. 2004; Mannucci et al. 2009; Zhang et al. 2009]. The incidence of hypoglycaemia increases with duration of diabetes and of insulin therapy [Henderson et al. 2003; Donnelly et al. 2005]. Hypoglycaemia has been considered a much more serious and common problem in type 1 diabetes[DCCT 1993; ter Braak et al. 2000] than in type 2 diabetes but if patients with type 1 and patients with type 2 are matched for duration of insulin treatment the hypoglycaemia rate differs little [Hepburn et al. 1993].

The SU used in **Study III** was glimepiride. This compound is associated with a lower frequency of hypoglycaemia than e.g. glibenclamide is [Holstein et al. 2001] and glimepiride is one of the two SU compounds recommended in Swedish guidelines [Läkemedelsverket 2010]. One caveat when comparing hypoglycaemia rates between studies is the lack of consensus on a common definition of biochemical hypoglycaemia [Service 1995]. Thus various glucose levels around 3 mmol/l have been defined as hypoglycaemia, and we use the same definition as was used in an earlier study [Holman et al. 2007] for both SMPG and CGMS. No major hypoglycaemia occurred during study III but adding glimepiride to insulin and metformin resulted in a higher frequency of minor hypoglycaemia, both when assessed as SMPG and as CGMS during nighttime, than placebo did. However, six of the 21 patients who reported minor hypoglycaemia with SMPG < 3.1 mmol/l, had only one episode during three months of glimepiride addition. Note also that all patients in **Study III** were on insulin and forty percent were treated with complex insulin regimens, using > 3 insulin injections/day: this has been associated with an increased risk of hypoglycaemia [Holman et al. 2007].

In previous studies long diabetes duration has been associated with an increased risk of hypoglycaemia [Henderson et al. 2003; Akram et al. 2006] but this was not found in **Study III**. In the stepwise regression model used to evaluate factors related to hypoglycaemia, diabetes duration was not related to its occurrence. However, older age was associated with a higher risk of hypoglycaemia, and low endogenous insulin secretion at baseline was also significantly associated with an increased risk. This is in line with other reports [Shorr et al. 1997; UK Hypoglycaemia Study Group 2007]. The incidence of hypoglycaemia has been related to the level of glucose control [Miller et al. 2001; Henderson et al. 2003], but in **Study III** $HbA_{1c}$, - neither baseline nor change or final level - did predict the occurrence of hypoglycaemia.

CGMS provides data on nighttime glycaemic profiles that are difficult to obtain with SMPG and thus CGMS could be valuable for detecting hypoglycaemia in type 2 diabetes patients. This is particularly important in view of the evidence suggesting that older people have fewer and less intensive symptoms of hypoglycaemia [Brierley et al. 1995] and that the difference, between the glucose level at which hypoglycaemic symptoms are generated and the onset of cognitive function, is lost in older patients [Matyka et al. 1997]. It is speculated that the aged brain is less able to perceive physiological and cognitive alterations associated with hypoglycaemia [Bremer et al. 2009]. Hypoglycaemia might go unrecognized in older people [McAulay et al. 2001], especially during nighttime, and this may be deleterious because recurrent low glucose values may result in impaired awareness of hypoglycaemia [Segel et al. 2002]. The impaired perception of hypoglycaemic symptom in elderly people increases the risk of severe hypoglycaemia [Schopman et al. 2011].

**Study IV** was performed in the early clinical era of CGMS and the durability of the sensor accuracy of nocturnal CGMS readings was studied for the first sensor model. CGMS was initially introduced as a useful tool to detect unrecognized nocturnal hypoglycaemia [Boland et al. 2001; Chase et al. 2001]. The accuracy and performance of the first sensor system were questioned by McGowan et al who documented that when comparing simultaneous glucose reference values with CGMS readings, CGMS generated lower nocturnal values in well-controlled type 1 diabetic patients [McGowan et al. 2002]. In most studies in which sensor accuracy was tested, no

confirmatory reference values were used, and the sensor accuracy was evaluated by agreement of paired data from capillary glucose values used for calibration and sensor reading. This procedure may overestimate the accuracy of sensor performance [Djakoure-Platonoff et al. 2003]. Whether sensor performance remained stable over time [Gerritsen 2000] was a crucial question. This was the background to study IV, and indeed, the results did show decreased sensor accuracy during the third night of registration.

After **Study IV** was published, new sensor products have been developed. The performance and accuracy of the Medtronic REAL-time CGMS, using the same sensor as was used in **Study III**, was investigated in a large study which showed sensor readings in close agreement with glucose meter values; and when stratifying for duration of sensor wear for up to 72 hours, the sensor performed well [Mastrototaro et al. 2008]. Some studies suggest that sensor performance remains stable over three to seven days [Guerci et al. 2003; Chlup et al. 2006; Wong et al. 2006]. In one study in which CGMS readings were compared to reference P-glucose values, sensor accuracy did not deteriorate over nine days of continuous use [Iscoe et al. 2012]. The accuracy of a sensor is lower in the hypoglycaemic range [Klonoff 2005]. However, when hypoglycaemia is induced in clamp studies, CGMS has accurately reflected P-glucose down to 3.3 mmol/l [Caplin et al. 2003] tracking changes in glucose very well [Monsod et al. 2002].

When CGMS recordings between midnight and 6 AM in study III were analysed, six patients had two or more consecutive values < 3.1 mmol/l during the last three nights with placebo and the median time spent at these levels was 40 min (5-105). At the end of glimepiride treatment 38% (15/39) of the patients had values < 3.1 mmol/l and the median time spent at low glucose levels was 45 min (5-280). None of these recordings were associated with symptoms. Asymptomatic hypo-glycaemic episodes during nighttime have also been reported from other CGMS studies in type 2 patients [Weber et al. 2007; Hanefeld et al. 2009]. A Spanish study reported that 43% of patients treated with insulin and oral agents had asymptomatic nighttime hypoglycaemia, detected by CGMS [Chico et al. 2003] and in an Australian report, where well-controlled ($HbA_{1c}$ 6.2%, IFCC 44 mmol/mol) older patients (mean age 74 years) on oral therapy including SU, were studied with CGMS, 56% patients had glucose levels < 2.2 mmol/l, mostly during nighttime and all unrecognized by the patient [Hay et al. 2003]. The duration of hypoglycaemia in the latter study was 78 min (20-305 min). Similar results were reported by McNally et al with at least one episode of glucose < 3.5 mmol/l at night in 51%-66% of patients in a study comparing two insulin regimens; and the majority of these events were unrecognized [McNally et al. 2007]. The conclusion is that well-controlled type 2 patients probably have a significant number of un-recognized hypoglycaemias during nighttime, but whether this reflects clinically relevant hypo-glycaemia could be debated. However, the use of CGMS in **Study III** provided information unobtainable with SMPG and allowed for therapeutic decisions in some patients that would otherwise not have been made.

To conclude, older patients with type 2 diabetes with long duration of insulin therapy, complex insulin regimens, and low endogenous insulin secretion [UK Hypoglycaemia Study Group 2007; Amiel et al. 2008] are at the same risk of developing hypoglycaemia as people with type 1 diabetes. Hypoglycaemic events in older people may be overlooked because of fewer symptoms perceived, further increasing the risk of severe hypoglycaemia. Against this background, CGMS

could be justified in a subgroup of patients with type 2 diabetes, selected as being at high risk of developing hypoglycaemia. However, the CGMS systems today are expensive for extensive routine clinical use and the technique may also be too complicated for older people to handle. Although sensor performance has undergone improvements, accuracy has been sparsely evaluated by comparing reference plasma glucose and sensor readings. More evidence regarding the usefulness of CGMS for type 2 diabetes is also needed, although small studies do show reduced frequency of hypoglycaemia and time spent at low glucose levels with the use of CGMS [Garg et al. 2006; Weber et al. 2007].

### Can SU accelerate the deterioration of β-cell function?

There has long been much concern that treatment with SU could hasten the deterioration in β-cell function. There are data showing that glibenclamide can induce β-cell apoptosis [Maedler et al. 2005], but there may be differences between SU compounds with respect to function and survival of cultured human islets. Thus, glibenclamide has more deleterious effects than glimepiride does [Del Guerra et al. 2005]. Gliclazide was shown in one study not to induce apoptosis [Sawada et al. 2008] or could even protect β-cells from apoptosis [Del Guerra et al. 2007]. These differences are probably related to the more transient binding to the SU receptor with glimepiride and gliclazide. However, whether these findings translate into in vivo conditions and are of clinical relevance is not clear. It has also been suggested that SU could stress β-cells by increasing insulin secretory demand, while elevated levels of IAPP [Rachman et al. 1998], the polypeptide involved in formation of islet amyloidosis, and of proinsulin, proposed to be a sign of stressed beta-cells, have been associated with SU therapy [Pfutzner et al. 2006].

A less discussed but important finding from the UKPDS was, that the observed loss of β-cell function, assessed by HOMA-B, occurred at the same rate regardless of treatment used, suggesting that none of the therapies evaluated in UKPDS were more harmful to β-cells and that factors other than treatment modality contribute to the process [UKPDS 1998]. In contrast, a possible harmful effect of SU on β-cells was put forward in the more recent ADOPT study, in which the glycaemic durability of rosiglitazone, metformin and glyburide monotherapy and changes in β-cell function, assessed with HOMA-B, were studied over four years [Kahn et al. 2006]. The deterioration in glycaemic control and decrease in HOMA-B was greatest in the glyburide group and the authors conclude that it was the more rapid loss of β-cell function that led to the increase in FPG and $HbA_{1c}$ (the difference in $HbA_{1c}$ between rosiglitazone and glyburide was 0.4%) in patients allocated to glyburide. However, taking into account the role of insulin and glucose in the formula of HOMA-B, an assumption could be that rosiglitazone, an insulin sensitizer, lowers FPG to a greater extent [Abbasi et al. 2011], which could partly, account for some of the difference in HOMA-B. After the ADOPT study was presented the interpretation of the results regarding SU have been questioned by some authors [Al-Ozairi et al. 2007; Yki-Järvinen 2007; Riddle 2010] giving a more positive view of the usefulness of SU.

A beneficial effect of insulin compared to SU on insulin secretion and glycaemic control was reported from a Swedish group [Alvarsson et al. 2003]. In newly diagnosed type 2 diabetic patients, early insulin treatment resulted in a more pronounced C-peptide response to glucagon

after one and two years as compared to treatment with a low dose of glibenclamide. The degree of glycaemic control between the groups varied, with lower $HbA_{1c}$ in the group treated with insulin, which complicate the interpretation of the observed changes in β-cell function.

In **Study II** long-term exposure to SU therapy and change in β-cell function were studied. The patients were classified into three groups depending on SU exposure: continuously during ten years, periodically or never. Relation to change in postprandial C-peptide/glucose was analysed in a regression model. The conclusion was that treatment with SU did not have any effect on change in β-cell function.

Adult patients that are antibody-positive, i.e. have latent autoimmune diabetes in adults (LADA), a form of type 1 diabetes that initially presents as a clinical type 2 diabetes [Landin-Olsson 2002]. LADA is characterized by a slowly onset, and these patients probably have a faster β-cell failure rate, increasing the likelihood of insulin requirement [Turner et al. 1997]. LADA prevalence among patients with a clinical diagnosis of type 2 diabetes varies between 10-20% [Pozzilli et al. 2001]. The optimal treatment for these patients has not been established, but SU is probably not advisable since they might promote β-cell destruction here, and the general recommendation is insulin therapy, which might better preserve β-cell function [Maruyama et al. 2008].

## WHO BENEFITS FROM SULPHONYLUREA AFTER MANY YEARS OF DIABETES?

In both **Study I** and **Study III** one of the aims was to identify clinically useful markers or predictors of long-term responsiveness to SU but no such predictors were found. These results are in line with the conclusion from several studies in the 1980's [Groop et al. 1986; Riddle et al. 1989] and 1990's [Ravnik-Oblak et al. 1995; Lev-Ran et al. 1998; Trischitta et al. 1998; Landstedt-Hallin et al. 1999], namely, that it is difficult to find clinical or metabolic variables that predict glycaemic response to combination therapy. A review article [Lebovitz et al. 1990] suggested that patients with a BMI 25-35 $kg/m^2$ with poor glycaemic control on modest or high doses of insulin and significant C-peptide secretion when stimulated by nutrients are likely to respond to SU. Interestingly, duration of diabetes *per se* is not mentioned as a negative fact in the review although it is generally held that SU therapy is not effective after many years of diabetes. The finding in **Study I**, that the factors related to preserved SU responsiveness were a long duration of diabetes and of SU therapy, support the notion that SU compounds can in fact be effective despite many years of diabetes. The participating subjects in **Study I** were patients who had been exposed to insulin and SU over a long period as their diabetes duration was in median 19 years, and they had been on a SU component for 15 years, combined with insulin for 10 years. The observation that long duration of diabetes and duration of SU therapy were both positively related to SU responsiveness, a result which is in concordance with findings from the SISU (Scandinavian Insulin-Sulphonylurea) study [Landstedt-Hallin et al. 1999]. The increase in FPG after SU withdrawal in **Study I** was more pronounced in patients with longer duration of diabetes indicating that SU was more effective the longer a patient had suffered from diabetes. This may seem to be a paradox. However, it could reflect the heterogeneity of type 2 diabetes and one possible explanation could be that patients with a slower rate of β–cell deterioration had been able

to manage longer on oral therapy. It could also reflect a selection bias, since it is possible that primary care physicians would not change a seemingly beneficial treatment in patients treated with SU for many years. Furthermore, patients with a lower insulin dose/kg at baseline in **Study I** had a more pronounced increase in FPG. A possible explanation for this could be that patients with lower insulin doses were more dependent on the SU compound for maintaining glucose control. In **Study III** older age was negatively related to response to glimepiride, which may relate to the general deterioration of β-cell function associated with aging [Chang et al. 2003; Scheen 2005].

Measurements of C-peptide would seem appropriate to use when trying to predict response to SU since a residual β-cell function is the prerequisite for a beneficial effect of SU therapy [Falko et al. 1985; Castillo et al. 1987]. However, in **Study III** it was found that the response to glimepiride was not related to fasting C-peptide or C-peptide/glucose and this is in line with earlier studies on SU combined with insulin [Riddle et al. 1989; Landstedt-Hallin et al. 1999]. The increment in C-peptide or ratio C-peptide/glucose - from fasting to postprandial - that reflects the ability of β-cells to increase insulin secretion could, at least, theoretically be more informative. When these values were evaluated in **Study III** the increment in C-peptide/glucose ratio at baseline was related to a positive response to glimepiride explaining 12% of the variance in $HbA_{1c}$ and the increment in ratio also related positively to glimepiride response in a Japanese study [Yokoyama et al. 2010].

To summarize long duration of diabetes and of SU therapy predicted a continued beneficial effect of SU, which lends further support to our earlier conclusion, namely that SU are effective in long-term combination with insulin. Neither C-peptide alone, the ratio C-peptide/glucose or the increment of the ratio may be considered clinically useful predictors of response to SU therapy in patients with long-standing diabetes.

## THE GLOBAL USE OF SULPHONYLUREAS – WHY DIFFERENCES?

Sulphonylurea compounds have been one of the most commonly used oral medications for glucose-lowering in type 2 diabetes worldwide. They were introduced more than half a century ago and it is unlikely that large studies focusing on these agents will be performed, especially now that newer treatments are coming onto the market.

The use of SU varies substantially between countries, with an apparent large variation in therapeutic traditions rather than in treatment guidelines. These almost always include SU as a treatment option [IDF 2006; Home et al. 2008; Socialstyrelsen 2010; ADA 2012; Inzucchi et al. 2012]. In the ongoing GUIDANCE study (Guideline Adherence to Enhance Care), which is seeking to assess the quality of care of people with type 2 diabetes and adherence to guideline recommendations, data from eight European countries, including Sweden have been collected [Stone et al. 2010]. The use of SU was lowest in Sweden with only 19% SU treated patients, compared to 46% in France and 33% in the whole sample [Khunti 2012]. The data for Sweden are confirmed by recent data from the Swedish National Diabetes Register, showing that approximately 18% of patients are treated with a SU compound [Ekström et al. 2012]. In many

developing countries SU therapy is probably more common. Thus in many African countries the use of SU has been reported to be around 60% [IDF 2008]. The many possible reasons for these differences including available resources for health care, national or regional prescribing traditions and perhaps also the influence of misconceptions about the utility of old drugs and proposed disadvantages e.g. that SU compounds accelerate β-cell loss.

In review articles on pharmacological treatment of type 2 diabetes it is stated that SU compounds are effective, safe, inexpensive [DeFronzo 1999; Del Prato et al. 2006; Bolen et al. 2007] and cost-effective [Gray et al. 2000]. In many less well-resourced countries the increasing costs for a growing number of people with diabetes and their care is a critical issue e.g. in the selection of drugs. The IDF Global Guidelines for the treatment of type 2 diabetes state that "in many parts of the world the implementation of particular standards of care is limited by lack of resources" [IDF 2006]. The IDF recommends metformin and SU and conclude that "cheap generic versions of these drugs are available, and their glucose-lowering capacity is not surpassed by any newer drug, at least on a population basis" [IDF 2006].

To summarize it is difficult to explain why there are such large variations in the use of SU but the differences do not appear to be based on their efficacy or cost.

## FUTURE PERSPECTIVES

The work presented in this thesis has tried to elucidate a few questions regarding the use of SU in type 2 diabetes, especially in patients with a longer duration of the disease. Mainly two issues have been addressed and they concern how we can increase the translation of research results into clinical practice to improve the medical care of patients with type 2 diabetes.

First, there is a considerable variation between individual patients in the loss of β-cell function over time and more studies are needed to investigate by what mechanism responsiveness to SU may be preserved over several years of treatment. The individual variation in the rate of β-cell loss makes the practice of routinely withdrawing SU in patients after many years of diabetes questionable. However a controlled period of withdrawal can give information about the usefulness of SU in an individual patient. It may also be worthwhile to try SU as an add-on therapy in patients who are not reaching their glycaemic goals on metformin and insulin.

Secondly, hypoglycaemia remains a limiting factor for achieving good glycaemic control of diabetes. Intensification of glucose-lowering therapy with insulin and SU in type 2 diabetes leads to higher incidence of hypoglycaemic events, many of which are undetected, and this may in turn provoke major vascular events in elderly patients [Zammitt et al. 2005]. Meticulous prevention of hypoglycaemia in patients at risk is therefore advocated and in this context the use of CGMS may prove helpful. Carefully conducted clinical studies are needed to assess the accuracy and efficacy of such systems in type 2 diabetes patients at risk.

# CONCLUSIONS

- Sulphonylureas are effective when combined with insulin even in patients with long-standing type 2 diabetes and there seems to be no rationale for routinely withdrawing the SU component after many years of combination therapy.

- β-cell function, assessed as the ratio of postprandial C-peptide/glucose, decreased over an observation period of ten years but long-term treatment with SU was not associated with are more pronounced decline in β-cell-cell function.

- Add-on therapy with glimepiride to insulin + metformin treatment in patients with long diabetes duration was effective in lowering $HbA_{1c}$ and/or reducing the need for exogenous insulin.

- Careful evaluation of nocturnal CGMS registrations in type 2 patients with reference plasma glucose assessments may be advocated to ascertain the accuracy of such readings over time.

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